AD

RESEARCH AND DEVELOPMENT ON INHALATION TOXICOLOGIC EVALUATION OF RED PHOSPHORUS/BUTYL RUBBER COMBUSTION PRODUCTS

PHASE I REPORT

CATHERINE ARANYI

AUGUST 1983

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701

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IIT Research Institute, Life Sciences Research Division
10 West 35th Street, Chicago, Illinois 60616

Contracting Officer's Technical Representative

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Inhalation exposure facilities for laboratory rats with automatically controlled conditioned filtered air supply and appropriate chamber exhaust through a coalescent filter system were built with five 1-m^3 exposure chambers. Red phosphorus/butyl rubber combustion generators provided by the Government were installed for each aerosol chamber. A separate room and air handling system were provided for two chambers to be used for exposure of control rats to filtered air. Aerosol sampling methods established for monitoring of the chamber atmosphere included measurements of mass concentration ...

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SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered) gravimetrically and optically, particle size by a quartz crystal microbalance-based cascade impactor and analysis of percentage phosphorus acid spectrophotometrically. Preliminary experiments were conducted testing generator performance and various RP/BR batches. Subsequently aerosol spatial and temporal homogeneity were tested in all chambers. Extensive statistical analysis of the pilot chamber revealed conditions of spatial and temporal homogeneity for filter and photosensor samples and for percent phosphoric acid levels. A statistically significant, spatial particle size gradient found, was not significant biologically in terms of inhalation and deposition into the lung. Particle size distribution was homogeneous when measured over time. Although inspection of four additional chambers revealed some significant deviations from a statistical point of view, these deviations were under the 20 percent variation limits *a priori* set for the homogeneity tests on the basis of physical performance of the complex test article-generator-chamber system. Therefore, we concluded that conditions in these chambers were also homogeneous.

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EXECUTIVE SUMMARY

The objective of these studies is to develop a data base for health hazard assessment of the effects produced by inhalation of combustion products from red phosphorus/butyl rubber used as an obscurant smoke for troops and vehicles in tactical and training environments. Laboratory rats exposed in inhalation chambers will be used to provide a comprehensive definition of the biologic effects of red phosphorus smoke to mammalian systems under conditions which approximate the potential troop exposure. This report summarizes the Phase I studies which were the establishment and standardization of the inhalation exposure conditions.

The inhalation exposure facility built for this project consists of conditioned air supply and chamber air exhaust systems; inhalation exposure chambers with air flow and differential pressure controls; and red phosphorus butyl rubber (RP/BR) combustion generators. Seven one cubic-meter inhalation chambers are available, five of which located in one laboratory are used for exposure to RP/BR aerosols. Two control chambers for exposure to filtered air are in a separate room. The combined exhaust air from the five aerosol-exposure chambers is filtered through a single-housing, 30-element coalescent filter and exhausted above the roof of the building. To prevent corrosion the carbon steel filter housing is coated on the inside with acid resistant polyvinyl chloride. A pressure differential gauge installed across the filter monitors saturation.

The aerosol was generated by burning RP/BR extruded through specially designed hydraulic extrusion-combustion generators provided by the U.S. Army Medical Bioengineering Research and Development Laboratory through Oak Ridge National Laboratory (ORNL). The RP/BR softened with hexane and prepackaged in stainless steel feed cylinders (billets), was also supplied by ORNL. The generator operates by exerting pressure through a hydraulic pump on the RP/BR contained in the feed cylinder. The material is forced by a piston to extrude from an orifice of the feed cylinder extending into the burn chamber of the generator and ignited by an electrically heated wire loop. The RP/BR is burned in and the combustion products are mixed with conditioned air and the aerosol is transported directly into the exposure chamber inlet port. At a constant chamber air flow rate the concentration of the aerosol is a function of the extrusion rate of the RP/BR which is controlled by the automatic hydraulic pump speed.

Throughout the studies temperature and relative humidity were monitored continuously and maintained between 24 to 27°C and 40 to 60 percent relative humidity. The

aerosol was monitored for mass concentration intermittently by filter samples and continuously with a photoelectric sensor, for particle size distribution with a piezoelectric microbalance, and for total phosphorus content by chemical analysis of the filter-collected aerosol samples. Oxygen concentration determined in the chambers during each test was consistently 21 percent. Chemical analysis of the chamber atmosphere indicated the absence of hexane, levels of less than 10 ppb of phosphine and variable, but relatively low levels of carbon monoxide, a maximum of 22 ppm, that could not be correlated with the RP/BR aerosol concentrations.

The objective of these studies was to evaluate spatial and temporal homogeneity of the chamber atmosphere in a three-dimensional array of points through a procedure of simultaneous sampling with cages and animal surrogates in place. For the pilot chamber, sufficient numbers of sampling points were selected to allow for characterization of spatial aerosol homogeneity within the chamber along with a series of sequential samples that were taken from a single or from multiple randomly selected fixed points to define temporal homogeneity for a period corresponding to the duration of the longest exposure. The aerosol was monitored for mass concentration, particle size and total phosphorus content at three generator settings (aerosol concentrations) replicating all tests at each generator setting three times. The ultimate objective was to reduce the variability of spatial and temporal homogeneity, with appropriate chamber modifications if necessary, to $\pm$ 20 percent of the mean of each parameter throughout the chambers and the range of concentrations tested.

Three test concentrations were selected on the following basis: the lowest operational concentration of the RP/BR generators at the 500 liter/min air flow rates used in our chambers (C1: 0.2-0.3 mg/l); the highest concentration that could be maintained for the 4-hr testing periods using the larger 0.75 in diameter RP/BR billets (C3: approximately 1 mg/l) and an intermediate concentration chosen between C1 and C3 (C2: approximately 0.5 mg/l).

After standardization of the pilot chamber was completed a single generator setting from those three evaluated for the pilot chamber was randomly selected for each of the four remaining chambers and spatial and temporal homogeneity tests were conducted in three replicate experiments for each chamber.

The statistical model used was a three factor mixed-modal analysis of variance. Concentration and location (shelf Nos. 1, 2, 3, 4 and center point) were considered to be the fixed factors, whereas replication was considered random; hence the term "mixed model". This model determines if between location differences are nonsignificant (there is spatial homogeneity) and if differences between locations depend on concentration (there is a concentration by location interaction). In the analysis of temporal homogeneity time was substituted for location as the second factor in the design.

Shelf means and individual sampling location levels were reported in percent mean deviation units from overall chamber means. Between chamber comparisons were made by comparing overall means and examining deviations between the parameters measured in the pilot chamber and each of the other chambers at appropriate concentrations.

The results demonstrated that the pilot chamber was spatially as well as temporally homogeneous in terms of aerosol mass concentration and percent total phosphorus and homogeneity was not affected by concentration. Particle size showed spatial heterogeneity and temporal homogeneity. However the overall range of 0.3 to 0.6 μm observed in particle size was such that this statistical significance was not biologically meaningful in terms of inhalation and deposition of particles.

To verify that the temporal and spatial homogeneity obtained in the pilot chamber were consistent in the other four chambers statistical analysis for each was performed. In addition maximum location deviations in terms of worst case shelf means were calculated for each of these chambers relative to the overall chamber means of each of the chambers. Although several of these statistical tests were significant indicating statistical heterogeneity the worst case deviation for all chambers was 17 percent from the overall chamber mean. Because of the large sample size the sensitivity of the statistical evaluation was beyond what could be required with the given physical limitations of the system. Thus under the 20 percent variation limit set as our goal, the data represented adequate levels of homogeneity.

For inter-chamber comparisons the overall means for each parameter and for each chamber were compared to the overall means of the pilot chamber for that respective concentration level. The data demonstrated that all between-chamber comparisons were within 16 percent of the pilot chamber for all measured parameters; hence it was concluded that the targeted concentration values were attained in the additional chambers.

Thus the extensive statistical analysis of the pilot chamber revealed conditions of spatial and temporal homogeneity for RP/RB aerosol mass concentration and for percent phosphoric acid levels. Although a statistically significant, spatial particle size gradient was found the variation was not significant biologically in terms of inhalation and deposition into the tracheobronchial region and the deep lung. Particle sizes were homogeneous when measured over time. Inspection of four additional chambers revealed some statistically significant differences; however, the worst case deviations for each shelf relative to its overall chamber mean and for each chamber relative to the pilot chamber were under the 20 percent variation limits set for the homogeneity tests on the basis of overall performance of the complex test article-generator-chamber system. Therefore it could be concluded that adequate levels of homogeneity were attained in all chambers.

FOREWORD

This report, IITRI No. L06139, Phase I Report describes studies conducted by the Life Sciences Division, IIT Research Institute for the Health Effects Research Division, U.S. Army Medical Bioengineering Research and Development Laboratory during the period of April 1982 through May 1983. The studies were carried out under Contract No. DAMD17-82-C-2121.

Catherine Aranyi served as Principal Investigator and James Fenters was Co-Investigator. The principal professional associate was Stanley Vana who was responsible for the inhalation exposure facilities, the aerosol generation and monitoring system and for conducting the aerosol homogeneity studies. Chemical analysis of various components of the chamber atmosphere was done under the supervision of Alan Snelson. Robert Gibbons, Consultant Biostatistician, and Narayanan Rajendran contributed to the sampling design for the chamber homogeneity testing. Statistical analysis of the aerosol homogeneity studies was performed by R. Gibbons.

Citation of commercial organizations and trade names in this report does not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

QUALITY ASSURANCE STATEMENT

Analytical Chemistry operations were inspected on six occasions between February 4 and April 12, 1983. The final draft report was audited between August 29 and September 3, 1983. Quality Assurance audits and inspections were conducted by Josephine Reed, Julie McPhillips and Kirit Parikh.

The study was found to conform to IITRI Life Sciences Quality Assurance criteria developed to meet FDA Good Laboratory Practice Regulations (Fed. Reg. CFR, Part 58, 1978). Raw data generated during the course of the study will be retained in the IITRI Life Sciences Archives.

Josephine M. Reed

Josephine M. Reed
Supervisor, Quality Assurance

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INTRODUCTION

As part of an overall concern for personnel health and safety, the U.S. Army Medical Research and Development Command is seeking to evaluate the effects produced by inhalation of combustion products from red phosphorus/butyl rubber used as an obscurant smoke for troops and vehicles in tactical and training environments. Laboratory rats, exposed in chambers will be used to provide a comprehensive definition of the biologic effects of red phosphorus smoke to mammalian systems under conditions which approximate the potential troop exposure. The approach to this research includes range- finding acute studies to determine lethal concentrations and influence of exposure duration on mortality; repeated exposure studies to define time-concentration relationships as well as threshold levels, healing, and adaptation in biologic reactions; and a subchronic exposure study with a recovery and observation period after the experimental exposure. The principal biologic response criteria to be monitored include overt toxic signs, clinical chemistry and hematology, histopathology, alveolar macrophage pulmonary defense functions, pulmonary bactericidal activity and neurobehavioral activity. The research project is set up to proceed in a phased manner. The objective of the Phase I studies was to set-up a government-supplied generation system to provide freshly generated combustion products from a mixture of red phosphorus and butyl rubber and to establish a suitable exposure facility for producing an inhalation chamber atmosphere from these combustion products. Chamber sampling techniques, physical and chemical monitoring procedures were standardized and aerosol homogeneity in the exposure chambers was tested.

I. FACILITIES

The major components of the inhalation exposure facilities are the conditioned air supply and the chamber air exhaust systems; the inhalation chambers with air flow and pressure controls; and the red phosphorus butyl rubber (RP/BR) generators. The facility is equipped with seven one cubic-meter inhalation chambers, five of which are located in one laboratory and are used for exposure to RP/BR smokes. Two control chambers for exposure to filtered air are in a separate room to prevent contamination. (See Appendix B, Figures B1-5).

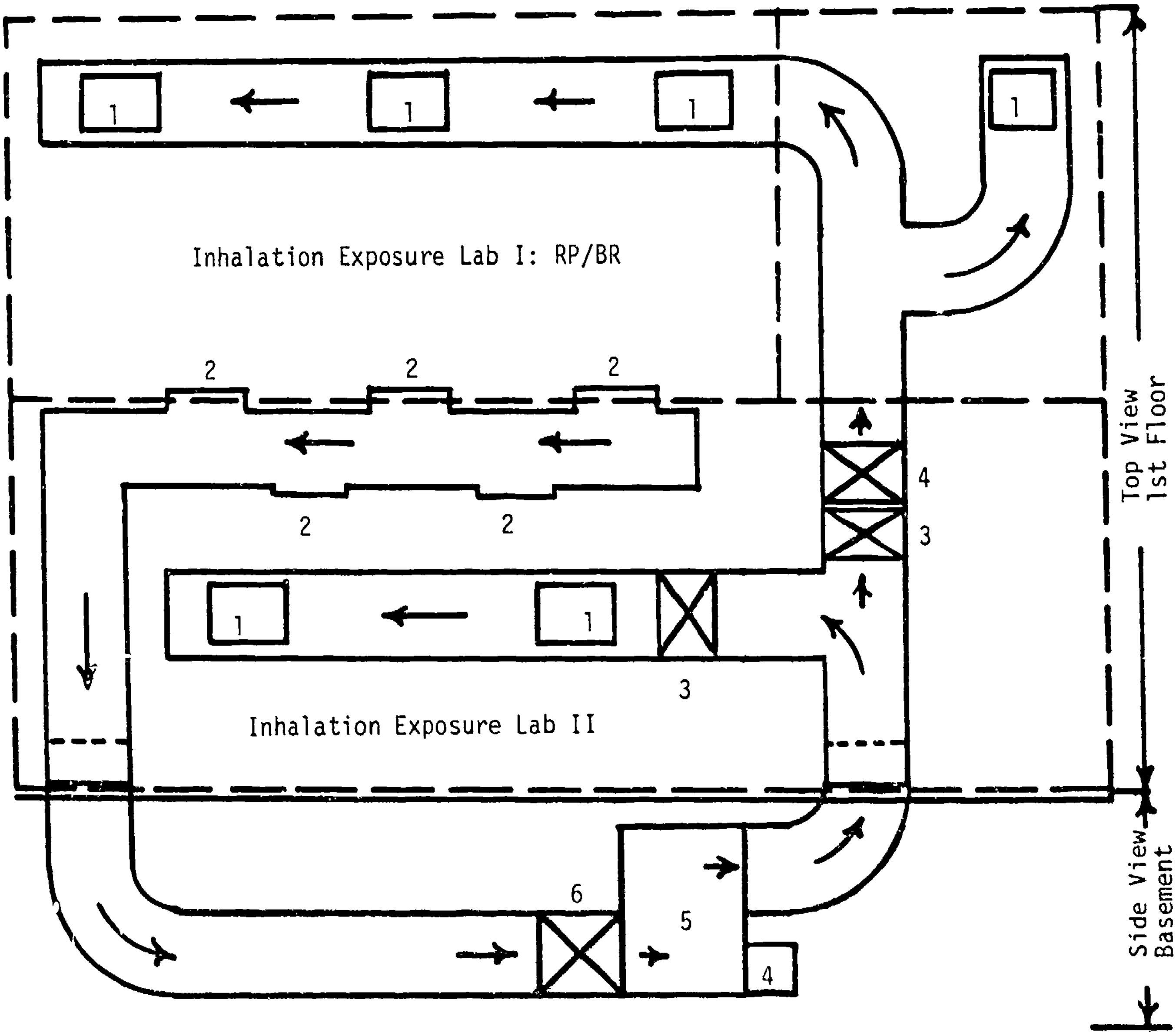
A. Supply Air

A schematic diagram of the air supply system is shown in Figure 1. Supply air passed through six 12 in x 12 in x 1 in prefilters (40 percent efficiency for 0.9 m particles) and six 11.5 in x 11.5 in x 0.5 in charcoal filters (grade 4 x 10, Type PBL) before entering the system is preconditioned with a 8.75 ton water cooled air conditioning unit. Temperature and humidity are adjusted to the required conditions of 24-27 C and 40-60 percent relative humidity (RH). An electric duct heater with an automatic control system is used to maintain the required temperature range. Humidity is supplied by two steam humidifiers, one located at the air conditioning unit outlet and another in the air inlet duct to the laboratory and is controlled with a high-limit, 85 percent, pneumatic modulating controller. An automatic air handling control panel for regulating cooling, heating and humidity is located in the RP/BR inhalation exposure laboratory.

The conditioned air is introduced into the room at the rate of 18 to 20 changes per hour. The conditioned room air is introduced into the chambers through individual inlet filter assemblies consisting of a fiberglass coarse filter, a charcoal bed and a HEPA filter.

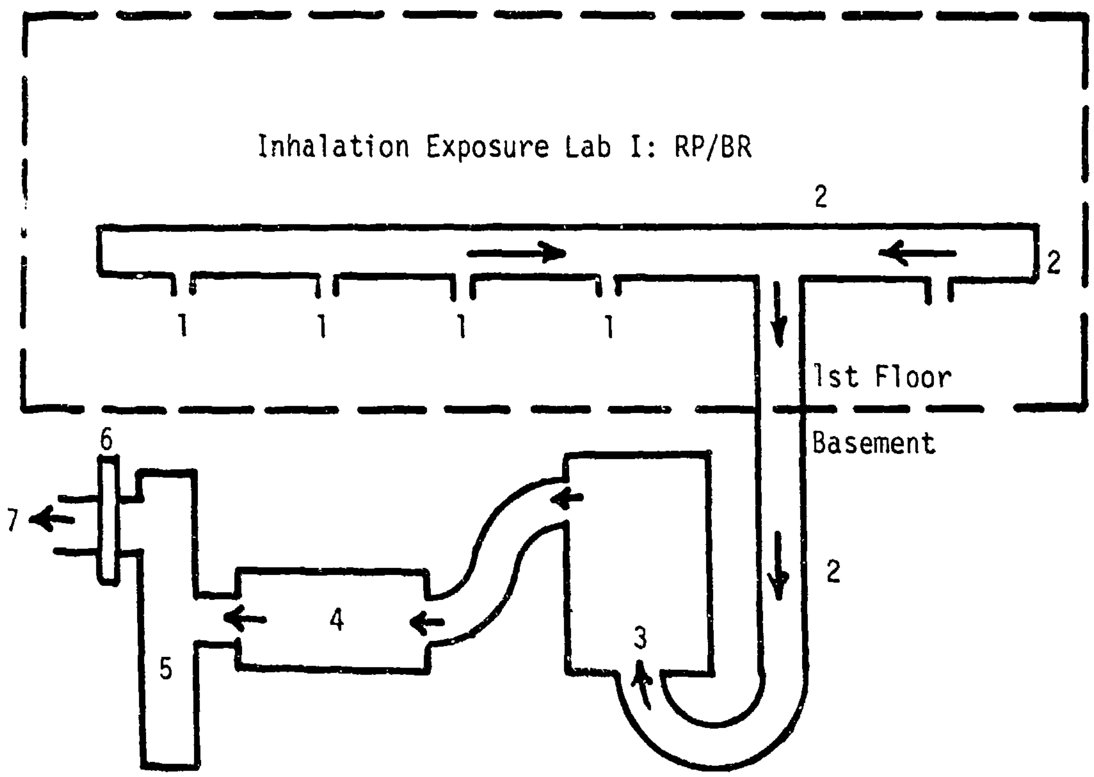
B. Exhaust Air

The experimental chamber air is exhausted through an 8 in. diameter spiral galvanized iron duct connected to the five experimental chambers with 3 in diameter flexible PVC ducting (Figure 2). The combined exhaust air from the five chambers is filtered through a single-housing, 30-element coalescent filter and exhausted outside the building above the roof. A pressure differential gauge installed across the filter monitors saturation.



1. Air supply vents
2. Air exhaust vents
3. Heater
4. Steam humidifiers
5. Air conditioning unit
6. Prefilter and charcoal bed

FIGURE 1: SCHEMATIC DIAGRAM OF THE CONDITIONED AIR SUPPLY SYSTEM



1. Chamber exhaust ports
2. Galvanized spiral ducting
3. Coalescent filter
4. Silencer
5. Blower
6. Damper
7. Exhaust to outside

FIGURE 2: EXPOSURE CHAMBER EXHAUST SYSTEM (Side View)

Originally a Model R-3080-8F carbon steel filter housing with 30 (No. 200-80-DX) filter elements from Balston Inc. was installed and used. By completion of the Phase I studies serious corrosion problems were encountered and the unit had to be replaced. The new unit consists of a modified carbon steel filter housing coated on the inside with an acid resistant polymer, polyvinyl chloride (PVC). A Balston filter housing, similar to the model originally installed, was purchased and modified at IITRI by cutting the unit into two sections and installing flanges with rubber gaskets for rejoining the sections. This modification allowed the entire interior of the housing to receive a continuous coating of PVC including the "double bottom" section. In addition, the portion of ducting connected directly to the filter housing inlet, where acid condensates have a chance to accumulate and cause corrosion has been replaced with a section of solid PVC. Inspection plates, installed at several points in the exhaust system ducting and monitored periodically for signs of corrosion have not shown any damage.

The exhaust air is moved by a pressure blower (2 HP, 3500 RPM motor) capable of providing > 500 liter/min airflow in each of the experimental chambers against 30 in of water pressure. A wafer type 6 in. damper serves for airflow control on the blower exhaust. A silencer filled with high density acoustical absorption material is installed between the blower and the filter. The air moving equipment is remotely located to minimize noise in the exposure laboratory. The blower is connected to an emergency power supply. In addition an alarm system installed in the exhaust air system provides warning in case of blower failure.

The exhaust system for the control chambers is independent of the system for the experimental test chambers to avoid potential contamination from the RP/RB. Both exhaust systems are operated continuously except during chamber cleaning or maintenance.

C. Inhalation Exposure Chambers

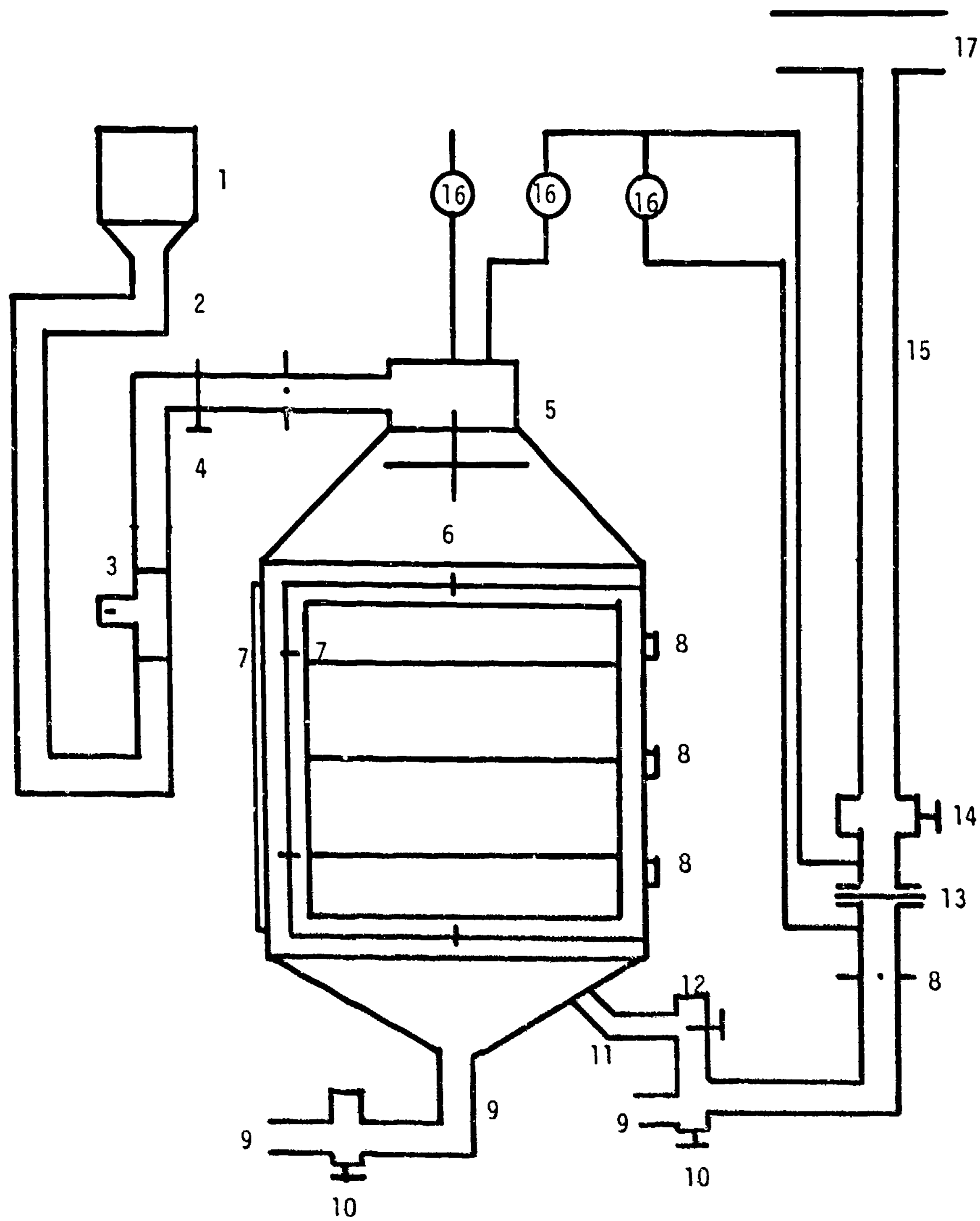
A total of seven (five experimental and two control) stainless steel inhalation exposure chambers are available for use. Each is approximately one cubic meter in volume which includes a central cubical (91 cm x 91 cm x 91 cm) and two pyramidal sections on the top and bottom respectively. A door is located on the front of the chamber. There are wire reinforced glass windows in the door and on one side wall of the chamber. Three sampling ports are located on the opposite side wall to the window. These ports were used for monitoring temperature and for collection of grab samples for carbon monoxide, hexane and phosphine.

The schematic diagram of an exposure chamber is shown in Figure 3. The RP/BR combustion products and dilution air are introduced into a mixing compartment located at the top of the chamber. An adjustable circular baffle plate located just below the mixing compartment aids in the uniform distribution of the test material in the main section of the chamber. A gate valve on the bottom is used for washing and drainage of the chambers after each exposure period. An additional gate valve is provided for draining water which may accumulate at the bottom of the baffle valve housing during chamber washing.

The chamber exhaust located in the bottom section, is a 2 in diameter tube with the opening facing downward. The exhaust passes through the wall of the chamber and has a baffle control valve box which is used for fine adjustment of the air flow rates. A 2 in diameter tube exits the valve box and has a flange section with an orifice plate for adjusting and monitoring total air flow rate. In addition total airflow through the chamber can be adjusted with a PVC valve located on the vacuum side of the orifice plate. Total air-flow rate is calibrated with a mass flowmeter and monitored by measuring the pressure differential across the orifice plate. A second gauge monitors the differential pressure between the chamber air inlet and exhaust to measure the total potential draw through the chamber. In addition, the negative pressure in the chamber relative to the room air pressure is continuously monitored with a differential pressure gauge.

D. The RP/BR Aerosol Generator

The aerosol was generated by burning RP/BR extruded through specially designed hydraulic extrusion-combustion generators provided by the U.S. Army Medical Bioengineering Research and Development Laboratory through Oak Ridge National Laboratory (ORNL). The RP/BR softened with hexane and prepackaged in stainless steel feed cylinders (billets) was also supplied by ORNL. The RP/BR is burned in and the combustion products are mixed with conditioned air (24-27 °C and 40-60 percent RH). The generator operates by exerting pressure through a hydraulic pump on the RP/BR contained in the feed cylinder. The material is forced by a piston to extrude from an orifice of the feed cylinder extending into the burn chamber of the generator and ignited by an electrically heated wire loop. As the RP/BR is extruded it burns at a generally uniform rate and the aerosol produced is transported directly into the exposure chamber inlet port. At a constant chamber air flow rate the concentration of the aerosol is a function of the extrusion rate of the RP/BR which may be controlled by the automatic hydraulic pump speed. A detailed description of the generator is provided in a publication from ORNL entitled "A System for



1. Inlet air filter
2. Flexible duct
3. RP/BR generator input
4. Valve
5. Mixing compartment
6. Deflection baffle
7. Reinforced glass windows
8. Sampling ports
9. Drains
10. Gate valves
11. Exhaust air line
12. Baffle valve
13. Limiting orifice
14. Exhaust air control valve
15. Flexible exhaust duct
16. Differential pressure gauges
17. Air exhaust duct

FIGURE 3. SCHEMATIC DIAGRAM OF STAINLESS STEEL INHALATION EXPOSURE CHAMBER

the Continuous Generation of Phosphorous Aerosols from Red Phosphorus-Butyl Rubber" by R. W. Holmberg and J. H. Moneyhun (Proceedings of Smoke/Obscurants Symposium VI, Harry Diamond Laboratories, April 27-29, 1982, Adelphi, Maryland). The schematic diagram of the RP/BR generator shown in Figure 4 is taken from this document.

Two alarm systems are available to alert personnel to potential malfunctions in generator operation. The light scattering photosensor system used for continuously monitoring RP/BR aerosol concentration in the exposure chambers can be set to a desired maximum concentration level above which it activates an alarm.

A monitoring device capable of detecting a flame-out of the extruded RP/BR was also installed at the generator burn chamber and operates by infrared monitoring of the flame. In the event of a flame-out of the RP/BR, it triggers an alarm alerting personnel to take immediate corrective action. Both devices were provided by ORNL.

E. Aerosol By-Pass and Dilution Systems

The operational method of the generator necessitates the use of a technique to protect the chamber atmosphere from high aerosol concentration surges during start-up or malfunction. Therefore a permanent by-pass valve installed at the chamber inlet was originally proposed. This emergency and start-up by-pass system was designed to divert the RP/BR aerosol from the generator before it would enter the exposure chamber. A three-way valve at the inlet to the chamber was to be manually or remotely activated, in the event of a malfunction in the generator or air handling system, to divert the RP/BR aerosol directly to the exhaust thus preventing exposure of animals to RP/BR aerosol concentration above levels specified, and/or allowing start-up of the generator in the by-pass mode until the burn rate became uniform and the target concentration had been reached.

Although this by-pass system prevented the RP/BR aerosol from entering the chamber, it had no provision for venting the chamber and thereby reducing the excess smoke already present. With the installation of the high and low aerosol concentration electronic alarms, having an aerosol by-pass system was no longer as essential as before. The "flame-out" alarm assures that interruption in aerosol generation cannot go unobserved. The high-concentration-alarm alerts operating personnel to any surge above the specified target concentration so the generator extrusion rate can be readjusted. In addition, a pre-burn is conducted prior to each exposure period to insure proper operation of the generator before animals are

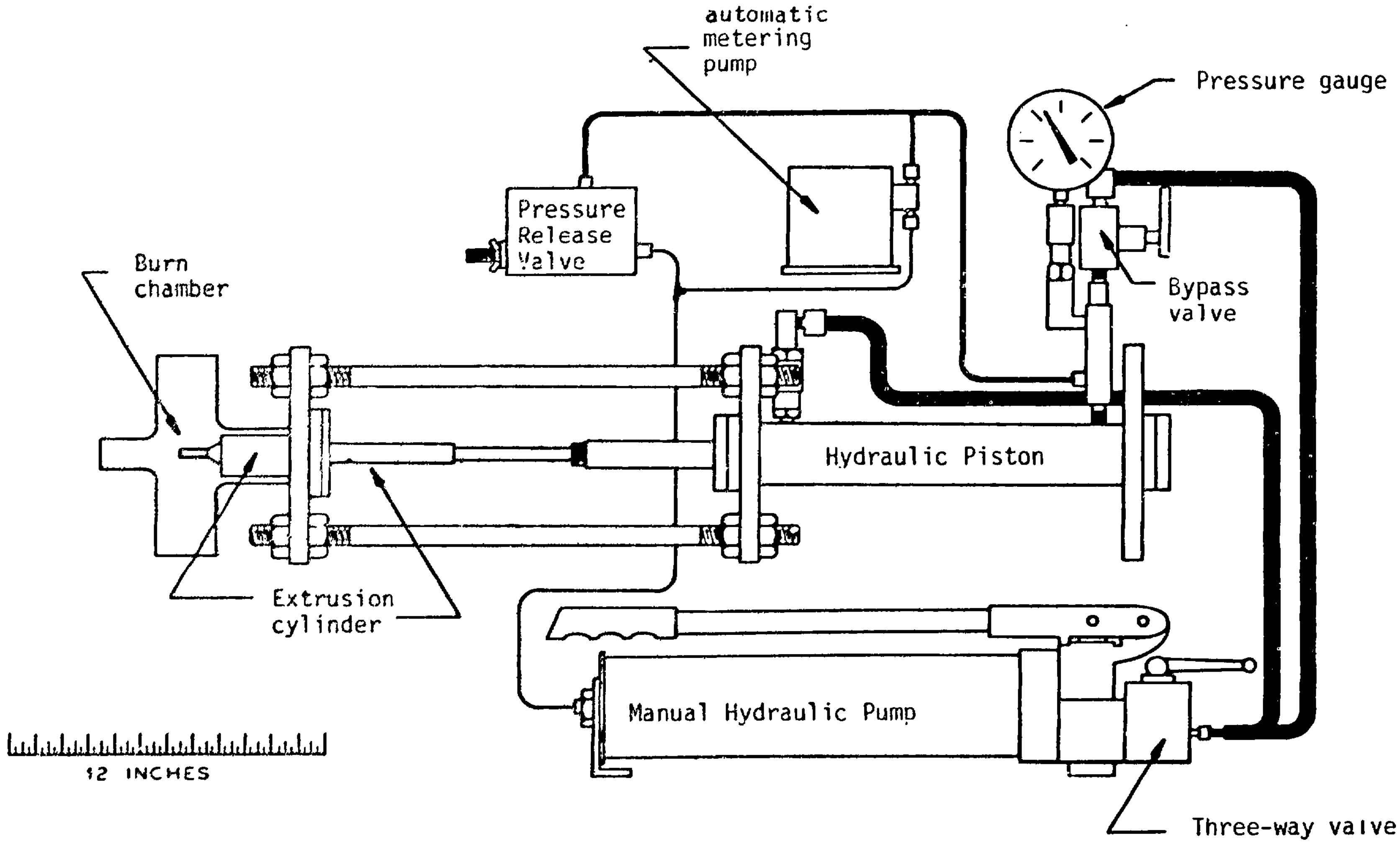


FIGURE 4: EXTRUSION - COMBUSTION GENERATOR FOR RP/BR AEROSOLS

From: R.W. Holmberg and J. H. Moneyhun
Proceedings of Smoke Obscurants
Symposium VI Harry Diamond Laboratories
April 27-29, 1982
Adelphi, Maryland

introduced into the chamber and the inhalation exposure is initiated.

For RP/BR aerosol concentrations lower than can be achieved with the generator, a dynamic di ution system was designed and a prototype unit was constructed for evaluation. The dilution apparatus with a by-pass valve was installed into the aerosol inlet duct between the exposure chamber and the aerosol generator (Figure 5). The dilution system shown in the schematic diagram of Figure 6 consists of two 'T's separated by a barrier and an inner connecting tube. The aerosol enters the system at P1 and exhausts through valve V1. Clean filtered dilution air enters the second 'T' through V2. The pressure at P2 is slightly less than the pressure at P1, so that a small portion of the aerosol is aspirated and diluted. The dilution ratio is determined by the flow rates of the aerosol through the inner tube and the dilution air through the second T. The system does not use any holding chambers and hence aging of the aerosols would not be a problem. However it may require additional study and modification because of potential airflow balancing problems.

In preliminary tests the system operated successfully when tested at a 1:2 dilution ratio between 0.8 and 0.4 mg/l aerosol concentrations. Further tests and development will be needed to assure that concentrations below 0.2 mg/l, the lower limit of generator capacity can be maintained for extended periods if necessary.

II. PRELIMINARY TESTS

A. The Test Article: Red Phosphorus/Butyl Rubber

The RP/BR test article softened with hexane was supplied by the sponsor in stainless steel 0.75 in diameter by 4.5 in long prepackaged feed cylinders, billets, with end caps. The airtight billets were stored in the laboratory at ambient condtions until used in the aerosol generator. Shipping dates, identification codes and specifications of the RP/BR, as reported to IITRI by ORNL, were maintained in a permanent record. The identification code and the number of RP/BR billets used in each study were entered in the experimental records.

The original shipments of RP/BR were received in 0.38 in diameter billets. However due to their short burning durations they were unsuitable for inhalation exposures and therefore they were replaced with larger 0.75 in diameter billets which approximaely doubled the burn duration. An adaptor was provided for loading the RP/BR from the large billets into the extrusion cylinder/piston assembly used in the aerosol generator. With this adaptor and the larger

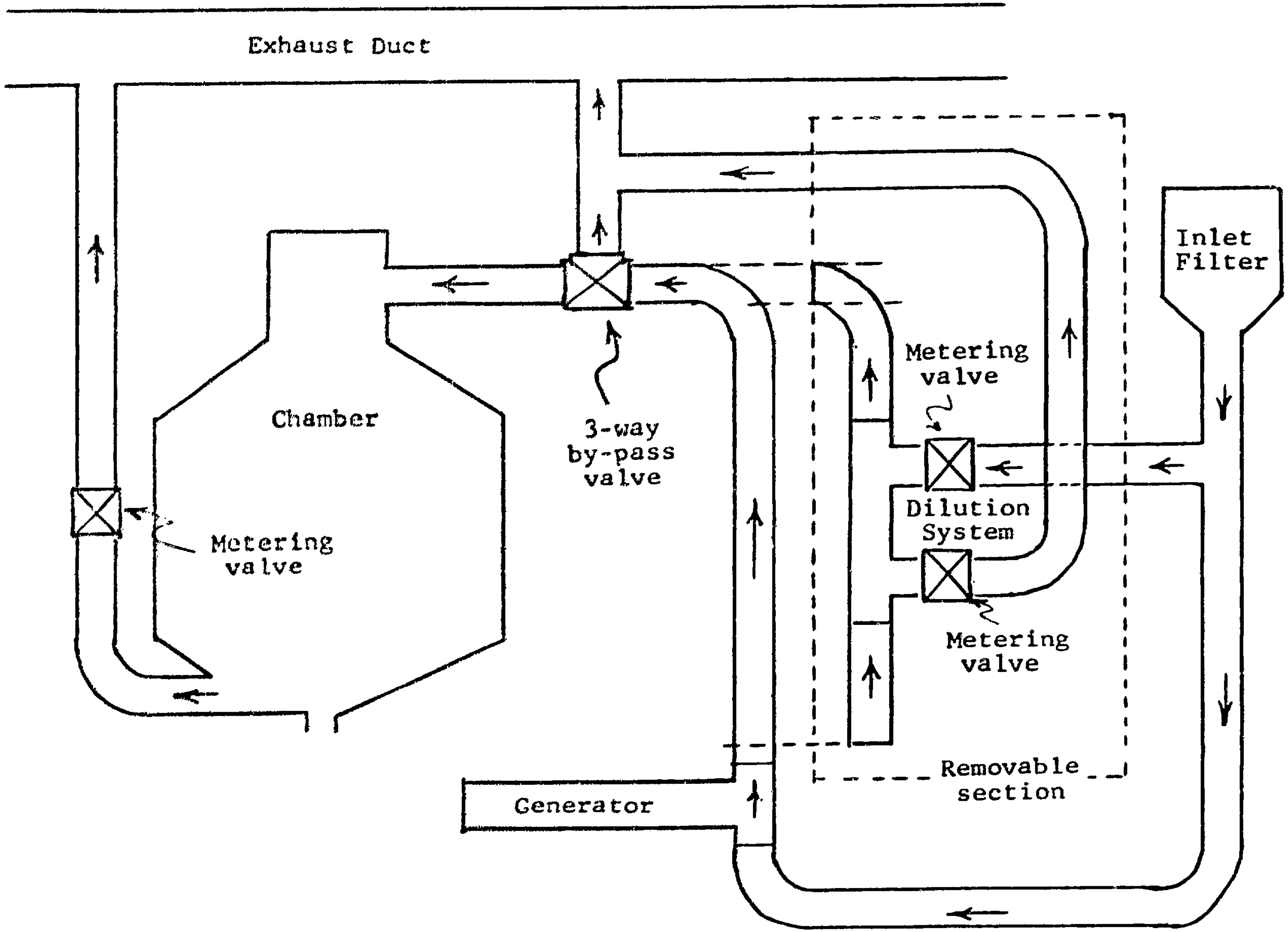


FIGURE 5: SCHEMATIC DIAGRAM OF AEROSOL CHAMBER BY-PASS AND DILUTION SYSTEM

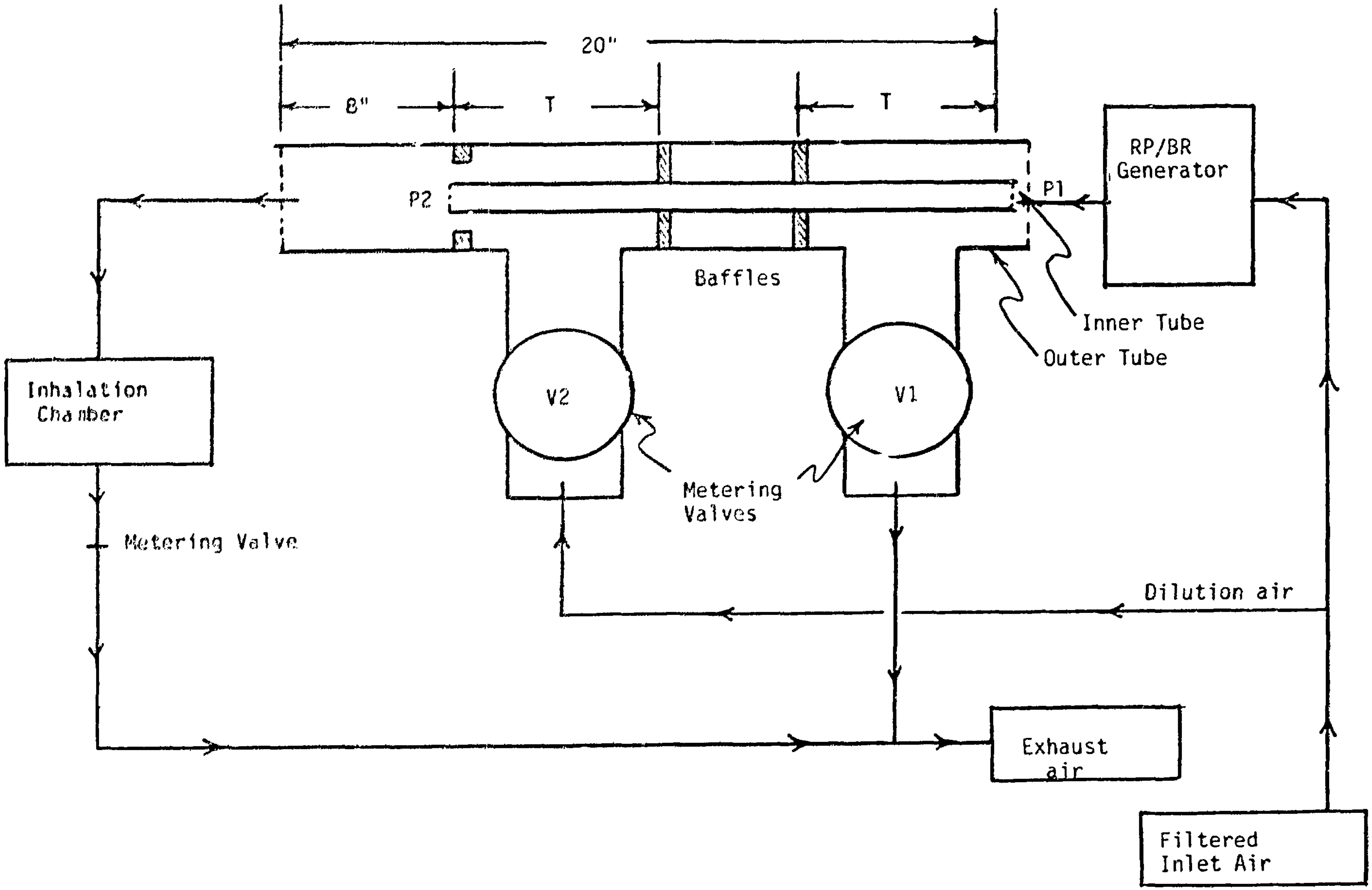


FIGURE 6: SCHEMATIC DIAGRAM OF AEROSOL DILUTION SYSTEM

billets, the entire extrusion cylinder could be loaded with RP/BR, whoreas previously with the 0.38 in billets it could only be partially filled.

B. The RP/BR Generator

The first RP/BR generator was delivered to IITRI in June 1982 from ORNL. It was assembled and its operation demonstrated by ORNL personnel. Subsequently it was used in exploratory aerosol generation tests until September when five new generators were received and the first one was returned.

During trial burn tests conducted with the generator in its original configuration it was found that the maximum achievable concentration (the maximum burn, or extrusion rate) was obtained with the automatic hydraulic fluid precision metering pump set at 0.150 in arbitrary units on the micrometer scale. Above this setting, the RP/BR was extruding faster than it was burning, and the material was advancing into the port of the burn chamber containing the ignitor coil. Under these conditions the RP/BR aerosol concentration was 2.8 mg/l and the burn duration 31 min. To increase the burn duration an attempt was made to charge two RP/BR billets into the extrusion cylinder. The generator operated for approximately 45 min and subsequently malfunctioned. When it was disassembled, it became evident that the breakdown occurred due to seizure of the piston in the extrusion cylinder due to galling.

The problem of short burn durations was later improved when the RP/BR was supplied in 0.75 in instead of 0.38 in diameter billets and an adaptor was provided for loading these larger amounts of material into the extrusion cylinder of the generator.

The new generators received in September from ORNL were installed behind each of the five inhalation exposure chambers on sturdy workbenches capable of supporting their weight and providing optimal access to support equipment and instrumentation. Care was taken to minimize the number of bends in the aerosol inlet duct leading from the generators to the chambers, thereby eliminating potential sites for particle deposition. The new generators were essentallly similar to the original in design except for a few modifications: The pressure gauge was replaced with a more sensitive model, (3,000 to 10,000 psi full scale); the large diameter flexible hose for the hydraulic pump hose was replaced with stainless steel tubing; the ceramic burn tip was replaced with a stainless steel tip and a newly designed ignitor was added. Most important was the change in the automatic hydraulic precision pump which on the new units could operate at lower extrusion rates and thus the generators could produce lower RP/BR aerosol concentrations. In addition, the new exrusion cylinder had a thicker wall, while its inside diameter remained unchanged. The extrusion piston was also redesigned so that only approximately 1 in at the front end matched the cylinder bore, the remaining portion was reduced in diameter to prevent "binding" between the piston and the cylinder.

These changes were made in part to correct some of the galling problems that were encountered during the preliminary burn experiments conducted with the original generator. Inspection of the defective units indicated scratches in the polished surfaces with normal use, even with extraordinary care in cleaning and handling. This may indicate the presence of abrasive material in the RP/BR. In order to decrease the occurance of galling the cylinder and piston were inspected after each use and polished if any signs of binding were encountered. In spite of these measures an occasional extrusion assembly is still lost because when severe galling occurs they cannot be salvaged by polishing.

C. Preliminary Burn Experiments

During the period when the generators were installed and the various RP/BR trial batches were prepared a series of exploratory experiments were conducted to determine the maximum and minimum RP/BR extrusion rates which would produce a relatively uniform and stable burn. In the first series of tests the original generator and the interim supply of RP/BR were used. To determine the relation between the automatic hydraulic pump performance and the burn characteristics at various airflow rates a billet (No. 012/8-23-82) was ignited and the generator automatic hydraulic pump speed gradually increased or decreased until the maximum or minimum extrusion burn rate of the RP/BR was obtained. At a chamber air flow of 500 liter/min the extrusion pump rates ranged from 0.300 to 0.020 in arbitrary units for the maximum and minimum burn rates attainable with the generator. This was determined by visual observation of the size and uniformity of flame pattern and the extruded material relative to the extrusion pressure and by observation of the burn characteristics (i.e. if the total extrusion was burning or just the distal part, and if the extruded material would break off and require re-ignition).

At the minimum burn rate, at an aerosol mass concentration of approximately 0.2 to 0.3 mg/l, the burn was relatively stable at a 500 liter/min airflow rate. However, residue accumulated at the burn tip which eventually interfered with the burn. Some flaring occurred and on occasion the build-up caused a flame-out. The residue appeared as an ash and as a viscous yellow material at the end of the tip.

When billet No. L2/8-19-82 was ignited at the maximum burn rate, a burn duration of approximately 30 min was obtained at an approximate aerosol concentration of 3 to 4 mg/l. At the 500 liter/min airflow rate the concentration stabilized relatively fast, however, some difficulty was experienced with the extruded portion breaking off or not burning uniformly, possibly due to cracks or variation in the RP/BR.

Subsequently the airflow rate was reduced to 250 liter/min. The extrusion rate was maintained as before and another billet (No. L4/8-19-82) of RP/BR was charged into the generator and ignited. At this lower airflow rate the burn was very erratic, the concentration increased gradually and did not stabilize by the time half of the material was burned. Also the temperature of the chamber inlet duct increased from the burning RP/BR and the generator burn chamber was quite warm. When the airflow rate was increased to 350 liter/min, the burn stabilized and aerosol mass concentration values ranged from 5 to 6 mg/l. Because of the large number of animals planned to be exposed per chamber, it was decided that a 500 liter/min flow rate, representing one airchange every two minutes in the one-m3-sized chambers, would be used in all future studies.

To examine burn stability and duration at various aerosol concentrations, 0.38 in diameter billets were burned at 500 liter/min airflow and at various extrusion rates to obtain targeted aerosol mass concentrations in the range of 0.2 to 3 mg/l. The results are summarized in Table 1. Experiment Nos. 1 to 4 were conducted with the first generator using the interim RP/BR supply. It can be seen that the increases in the settings of the hydraulic fluid precision metering pump are consistent with the increases in the targeted aerosol concentrations, whereas the required extrusion pressures varied with the various billets used. Aerosol mass concentration values determined (at various intervals after ignition of the RP/BR) gravimetrically on filter-collected samples and by continuous monitoring with light scattering sensors (calibrated at ORNL) were generally in agreement. Spectrophotometrically analyzed total phosphorus content from the filter collected samples and expressed as percentage phosphoric acid (H_3PO_4) in the aerosol are also shown in the Table. (Chamber sampling methods are reported in section III).

The continuous strip chart recordings of the photosensors showed deviations ranging to ±15 percent resulting from apparent nonhomogeneous RP/BR billets with

Table 1: EXPLORATORY BURN STUDIES WITH RP/BR BILLETS[1]

Exp. No.	RP/BR Billet No.	Extrusion: Pump Setting	Extrusion: Pressure (psi) min	Extrusion: Pressure (psi) max	Burn Duration (min)	Sampling Time (min): After Start	Sampling Time (min): Duration per Sample	Aerosol Mass Conc. (mg/l) from: Photo-sensor[3]	Aerosol Mass Conc. (mg/l) from: Filter Sample	% H_3PO_4 in Aerosol
1[2]a	01/8-23-82	0.125	600	1500	45	8		1.9[4]	1.89	-[5]
						23		2.2[4]	1.96	-
1 b	02/8-23-82	0.125	600	1400	45	8		2.0[4]	-	-
						30		2.1[4]	1.83	-
2	L5/8-19-82	0.020	700	1600	225	10		0.22	0.31	71
						35		0.22	0.32	68
						60		-	0.30	63
						83		0.18	0.27	70
						107		0.24	0.33	76
						143		-	0.33	76
3[2]a	L6/8-19-82	0.075	700	1000	70	28		0.91	-	-
						47		1.01	-	-
						53		0.96	1.03	85
3 b	L8/8-19-82	0.075	700	1500	68	27		1.04	1.14	69
						54		0.94	1.13	89
4	L9/8-19-82	0.040	800	2000	140	15		0.51	-	-
						24		0.41	-	-
						34		0.55	-	-
						49		0.51	-	-
						64		0.50	-	-
						85		0.52	-	-
14	Y1/11-10-82	0.250	250	350	70	10	12	3.14	3.05	-
						-	-	3.34	-	-
						42	11	3.29	2.96	-
						58	11	3.20	2.78	-
15	Y3-11-10-82	0.175	400	500	109	20	19	2.15	2.17	-
						42	13	2.07	-	-
						57	20	3.05	2.01	-
						81	20	2.07	2.11	-
21	Y4-11-10-82	0.100	450	1300	228	42	17	0.99	1.18	-
						67	12	0.94	1.15	-
						103	13	0.94	1.18	-
						140	13	0.89	1.11	-
						165	12	0.95	1.21	-
						182	12	1.02	1.24	-
						206	12	1.01	1.25	-

[1] Experiment Nos. 1-4 interim RP/BR supply, in 0.38 in billets, experiments 14, 15 and 21 permanent supply, in 0.75 in billets.

[2] There was a 30 and a 42 min interval respectively between phases a and b of Experiments 1 and 3 to recharge the generator with RP/BR.

[3] Adjusted to read 1 mg/l at 10 mV.

[4] From readings observed during the filter collection period. All following observations were integrated signal averages for the filter collection period.

[5] Not done.

hard spots. Burn duration for one billet was approximately 45 min at 2 mg/l, 70 min at 1 mg/l, 140 min at 0.5 mg/l and 225 min at 0.2 mg/l aerosol mass concentrations.

When the final standardized lots of RP/BR were received from ORNL tests conducted utilizing the larger 0.75 in diameter billets at aerosol concentrations of 3, 2 and 1 mg/l resulted in burn durations of 70, 109, 228 min respectively (Table 1, Experiment Nos. 14, 15 and 21). These tests were conducted in the new generators and comparison of the extrusion pump settings to those listed in the table for the first generator for similar target concentrations demonstrates the change in the precision pump performance in the new generators.

III. CHAMBER SAMPLING METHODS

A. Aerosol Mass Concentration

1. Gravimetric Method:

Aerosol mass concentration was monitored gravimetrically approximately once for each 2 hr exposure period. Particles of the RP/BR aerosol were collected on pre-weighed 45-mm fiberglass filter disks placed in acrylic plastic filter holders. The filters have 99.99 percent retention efficiency for dioctyl phthalate particles of 0.3 um. Prior to use the fiberglass filters were maintained for 24 hr in the conditioned atmosphere of the sampling environment to assure moisture equilibration by the filter pads. The aerosol samples were collected at constant flow rates of 2 liter/min using diaphragm-type vacuum air pumps. The filters were weighed on an analytical balance. Dry gas meters connected to the backside of the pumps recorded the corresponding total volume of air sampled.

All filter samples were weighed within 30 min of removal from the sampling ports, transferred to plastic petri dishes, and entered into a permanent record. Selected samples were subsequently submitted for total phosphorus analysis.

For aerosol homogeneity testing the chamber doors were temporarily replaced with a specially constructed plastic panel fitted to the front of the exposure chambers. A series of holes drilled into the plastic provided access for tubular stainless steel sampling probes 39 in long and 3/8 in in diameter to pre-determined sampling locations inside the chamber. The filter assembly was connected to the end of each probe outside of the chamber. This design assured uniform sampling of the aerosol and also that the aerosol samples always traveled the same distance from the sampling point to the collecting filter. (For detailed description of sampling locations see Section IV).

Filter weight stability To insure accurate sample weight determinations of the filter-collected aerosol samples, tests were conducted to evaluate the stability of filter weights over time, the effects of varying percent RH and the efficacy of isolating the filters from ambient atmosphere by sealing the ends of the holders with stoppers. Using a portable analytical balance, tests were conducted to determine the stability of filter weights over increasing time periods in the controlled conditioned air of the inhalation exposure laboratory. The results indicated that under these conditions the weights remained stable for one hour after collection. However the permanent location of the analytical balance is in an adjacent laboratory without conditioned air supply. Since the temperature and percent RH of the two laboratories was significantly different, tests were conducted to determine the effects on sample weight stability when filters collected in conditioned air (26 °C and 44 percent RH) were weighed at ambient conditions (20° C and 20 percent RH). To minimize the effects of this transfer the filter holders were sealed with silicone rubber stoppers which were removed during the actual weighing process only. Aerosol samples were collected on three groups of filters: in Groups 1 and 2 filter housing ends were left open and in Group 3 they were sealed with the stoppers. Each group was weighed at various intervals up to 1.5 hr after sample collection. Group 1 and 3 filters were weighed at ambient conditions and Group 2 in the controlled environment. The results indicated that the Group 3 conditions with stoppers on the filter holders produced the greatest sample weight stability. Group 1 samples collected at higher and weighed at lower percent RH decreased in weight over the total time observed, whereas Group 2 filters which remained at the higher percent RH generally increased in weight indicating moisture desorption or adsorption respectively by the filter pads and/or the collected aerosol particles. These tests demonstrated that the method producing the greatest weight stability was sealing the filter holders and weighing within 60 min after collection. This method became the standard operating procedure.

2. Light Scattering Method

Aerosol mass concentration was monitored continuously in each chamber with light scattering sensors. Permanent records of the amplifier outputs were maintained using strip chart recorders. Integrated averages of the photosensor values were taken simultaneously with the filter collection periods. The photosensor probes and amplifiers were provided and initially calibrated by ORNL. Subsequently, after having received a document on operation, maintenance and calibration of the units from ORNL they were recalibrated in our laboratories.

A brief description of probe and instrument and the principle of operation is quoted from "ORNL Aerosol Particle Sensor Description. Operation and Calibration" by J.H. Moneyhun, T.M. Gayle and R.W. Holmberg.

> "The ORNL/Gayle aerosol particle sensor system consists of a light scattering sensor and an electronic Read-out Module. The sensor is a commercially available (Optron OPB-710) assembly consisting of a light emitting gallium arsenide diode mounted directly beside a high gain phototransistor. The package is about 1/4 in. in diameter and height. The LED emits light in the near infared region (ca. 900 nm) which scatters from aerosol particles in the vicinity and is detected by the phototransistor. The Read-Out module contains the circuitry to power the LED and to condition, amplify and display the signal from the phototransistor. The signal is displayed on a digital meter and can be routed in analog form to a chart recorder. An integrating system with separate digital display is also provided so that sums or integrals of rapidly changing signals can be processed. Each sensor has its own characteristic sensitivity and must be individually calibrated. Our experience with a number of aerosols has shown that once calibrated they maintain their sensitivity for a long period of time providing their face is cleaned periodically to remove deposited particulate matter. Vernier adjustments are available so that the signal (the digital meter reports signal level in millivolts) can be made to correspond directly to aerosol concentration. While the response is not strictly linear, often, particularly over a restricted range of concentration, it is near enough linear so this "direct read out" can be utilized reliably without graphic interpolation. Typically we calibrate and adjust the gains so that 10 mV corresponds to a 1 mg/L aerosol concentration. The calibrations are made by comparing the output of the sensor system with the weight of aerosol collected on filter pads."

For homogeneity testing, photosensor sampling probes were also fitted to the chamber interior through the plastic front panel previously described. The light scattering sensors were placed inside of the probe tubes and positioned with the sensor extending slightly beyond the end of the tube. Adjustments in positioning the probes were made to minimize any back scatter reflections of sensor-emitted light from the exposure cages and chamber walls.

In addition to monitoring the RP/BR aerosol concentration, the photosensor system designed by ORNL has a built-in provision which can be set at any concentration level to activate an electrical alarm. This alarm alerted personnel to a malfunction in the exposure system indicating that there was a substantial deviation above or below the target concentration.

Standardization of Photosensors The light scattering aerosol sensors were standardized to RP/BR aerosol mass concentration values determined by the gravimetric filter sampling technique previously described. The approach to calibrating and adjusting the photosensor response was to introduce the RP/BR aerosol into the chamber and allow the concentration to stabilize as indicated by the photosensor response. A filter sample of the aerosol was then collected and concurrently an integrated value of the sensor response in millivolts divided by the sampling time in seconds was determined. The photosensor amplifier module, that had been originally adjusted to read 10 mV at 1 mg/L aerosol concentration, was then adjusted to correspond to the aerosol concentration as determined from the filter-collected sample. The output of the photosensor could then be used as a "direct" readout of the aerosol concentration providing continuous on line monitoring.

A typical standardization procedure of five photosensors simultaneously is summarized in Table 2. The five photosensors were placed in the chamber on one shelf along with a filter sampling probe. Filter samples were collected for four sequential periods with five concurrent integrated photosensor readings. After Set Nos. 1 and 2, the photosensor span was proportionally adjusted to correspond with the aerosol concentration as determined from the filter sample. Two additional sets were taken to confirm proper adjustment.

Table 2: PHOTOSENSOR STANDARDIZATION

Set No.	Aerosol Mass Concentration (mg/l) Determined by[a] Filter Samples	Photosensor Nos: 1	2	3	4	5
1	1.27	0.88	1.17	1.05	1.08	1.20
2	1.28	0.83	1.16	1.04	1.00	1.20
3[b]	1.30	1.23	0.96	1.20	1.15	1.27
4	1.29	1.30	1.19	1.18	1.26	1.36

[a] RP/BR aerosol concentration was determined over 20 min collection periods for filter and photosensor samples respectively.

[b] Photosensor amplifier adjusted before set No. 3.

Photosensor stability. In the beginning of each experiment the photosensors were set to zero before introducing the aerosol. Room lighting was kept at a minimum level and Mylar sheets were attached to the chamber windows.

Prior to the homogeneity tests, with the photosensors at the specified chamber locations, the amplifiers and chart recorders were set to zero. The effect of random room light was then determined by switching off various combinations of overhead lighting fixtures. When all lights in front of and directly over the chamber were turned off there was no observable difference in the readings compared with those obtained in total darkness. However, when the photosensors were zeroed at a given set of random positions and subsequently moved to other positions, the readings changed drastically with the slightest shift, requiring the amplifier dials to be reset to zero. Thus the photosensors were extremely sensitive to movement. Therefore, although our modification of the chamber door for homogeneity testing permits moving of the sampling probes during continuous operation of the generator, the photosensors were used in fixed positions since the amplifiers could not be reset to zero in the presence of the aerosol.

B. Aerosol Particle Size

Aerosol particle size distribution was monitored by a piezoelectric microbalance-based 10-stage cascade impactor. The Quartz Crystal Microbalance (QCM) is a cascade of aerodynamic-inertial impactors, in which the suspended particles are classified according to their effective aerodynamic sizes and weighed *in situ* and in real-time on the impaction surface. This is accomplished by using high-frequency, resonating piezoelectric crystals as the impactor plates. A built-in pump samples an aerosol stream at a rate of 0.24 liter/min, separating the aersol particles into 10 sequential size ranges from 0.05 to 25 um. Ten audio frequencies, which are proportional to the accumulated mass on the stages, are displayed and printed directly from the instrument. A built-in computer converts the data to the actual mass and size readings. Mass mean aerodynamic diameter was calculated for each sampling point from the corresponding mass fraction of particles accumulated on each stage of the QCM using a programmable calculator.

Particle size distribution measurements of the highly concentrated aerosols were accomplished with use of a sliding valve as shown in Figure 7. The sample is continuously drawn from the chamber and when particle size

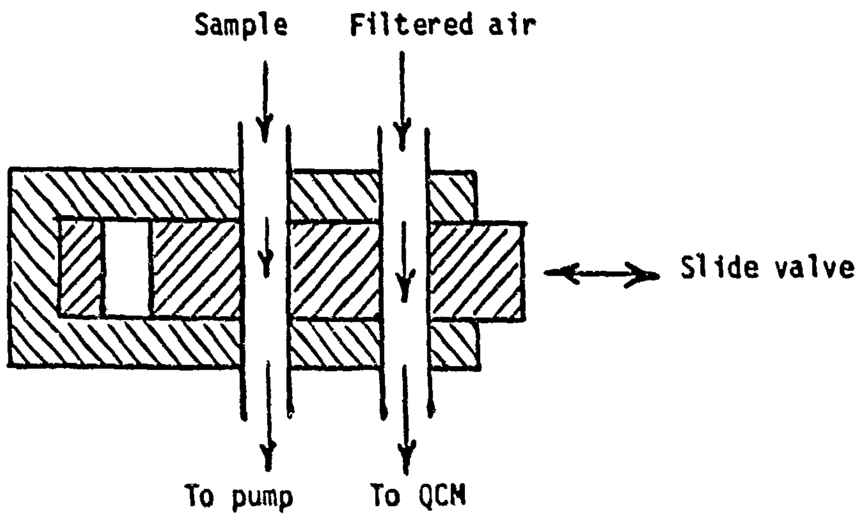


FIGURE 7. SAMPLING VALVE FOR QUARTZ CRYSTAL MICROBALANCE

is to be measured the slide is pulled out and a slug of the sample is drawn through the QCM. For homogeneity studies of aerosol particle size, samples were collected from the chambers sequentially from the same sampling probes provided for gravimetric filter sample collection.

C. Total Phosphorus Analysis in Filter Collected Aerosol Samples

Samples collected from the RP/BR aerosol exposure chamber for determination of aerosol mass concentration (see above) on tared glass fiber filters were analyzed for total soluble phosphorus. The method used was a modification of the Vanadomolybdophosphoric acid colorimetric procedure described in "Standard Methods for the Examination of Water and Wastewater", 14th Edition 1975 APHA-AWWA-WPCF. Briefly: In a dilute orthophosphate solution, ammonium molybdate reacts under acid conditions to form a heteropoly acid, molybdophosphoric acid. In the presence of vanadium the vanadomolybdophosphoric yellow color is formed. The intensity of the yellow color is proportional to the phosphate concentration in the solution. The minimum detectable concentration is 0.2 mg/l phosphorus in a 1 cm-long spectrophotometer cuvette.

A critical step in the application of the procedure is the hydrolysis/oxidation of all phosphorus species present on the filter to the phosphate ion form. Previous studies in this laboratory have indicated that a phosphorus (white phosphorus/felt wedge) smoke aerosol probably consists of a mixture of polyphosphoric acids. Organophosphorus compounds, if present, are at negligible levels in terms of total phosphorus present in the form of acid species. Polyphosphoric acids are readily converted to the phosphate form by boiling with nitric acid prior to the colorimetric determination. However, if phosphorus species are present which are fairly resistant to oxidation, the effectiveness of the nitric acid hydrolysis oxidation procedure may not be sufficient. To test the suitability of the nitric acid procedure, sodium phosphite ($Na_2HPO_3 \cdot 5H_2O$) was assayed (phosphite is known to be fairly resistant to oxidation to phosphate). Indeed the nitric acid oxidation/hydrolysis procedure was found to be ineffective. A procedure using a two-stage oxidation, first with hydrogen peroxide and then with nitric acid was found to be suitable providing all traces of H_2O_2 were removed prior to forming the vanadomolybdophosphoric acid complex. A small amount of manganese dioxide was found to be effective in destroying all H_2O_2 in solution.

The digestion-oxidation procedures using the standard reagent HNO_3+H_2SO_4 and the two step H_2O_2/HNO_3 process were applied to a potassium monophosphate (KH_2PO_4) and a sodium phosphite (Na_2HPO_3) standard solution respectively. Identical concentration versus absorbance curves were generated using both techniques (Figures 8 and 9). Finally, the digestion-oxidation procedure was applied to unknown aqueous solutions of phosphate and phosphite samples supplied by our Chemistry Quality Assurance Officer and analyzed. The results of these analyses, indicated the modified analytical procedures to be acceptable with an estimated accuracy of ±5 percent.

However in the exposure chamber analyses of total phosphorus, the procedure involved some additional steps: the digestion of the filter sample and a colorimetric correction for a color which developed due to the presence of a small amount of dissolved silica (from the filter) in the sample as a result of the digestion procedure. Thus the ultimate accuracy of the test was generally close to ±10 to 15 percent

D. Temperature and Relative Humidity of the Conditioned Air

Humidity of the chamber intake room air was monitored continuously by a dew point hygrometer. The hygrometer is a line operated, precision instrument for measuring dew point temperature, ambient temperature and water vapor pressure, with direct dial readout. This is accomplished by using a lithium chloride dew point probe and an ambient thermistor probe. A dual channel recorder is used to obtain linear outputs of voltage versus temperature for continuous and simultaneous recording of both ambient and dew point temperatures. Instrument readings of ambient and dew point temperatures and vapor pressures are taken periodically and percent relative humidity values are calculated according to the following formula

$$\text{Percent RH} = \frac{P_{H_2O}}{P^{\text{amb. tempr}}_{\text{sat, } H_2O}}$$

The data are reported as percent RH and °C ambient temperature averaged over one day periods.

The performance of the dew point probe was checked by measuring the dew point temperature of water saturated environment and comparing it to the actual dew point measured with a thermometer. The two dew point temperatures

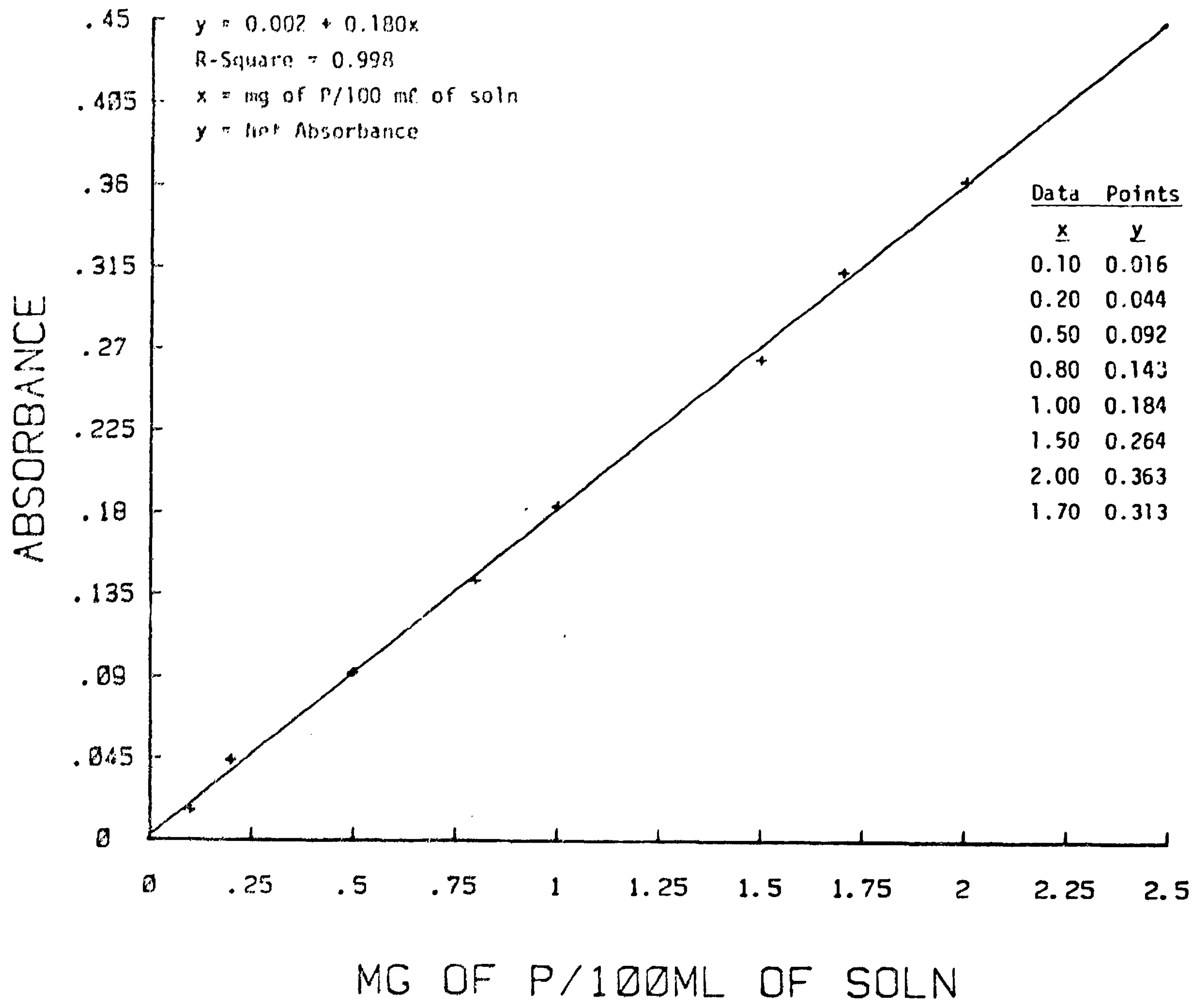


FIGURE 8. TYPICAL PHOSPHORUS CALIBRATION CURVE BASED ON A KH_2PO_4 STANDARD

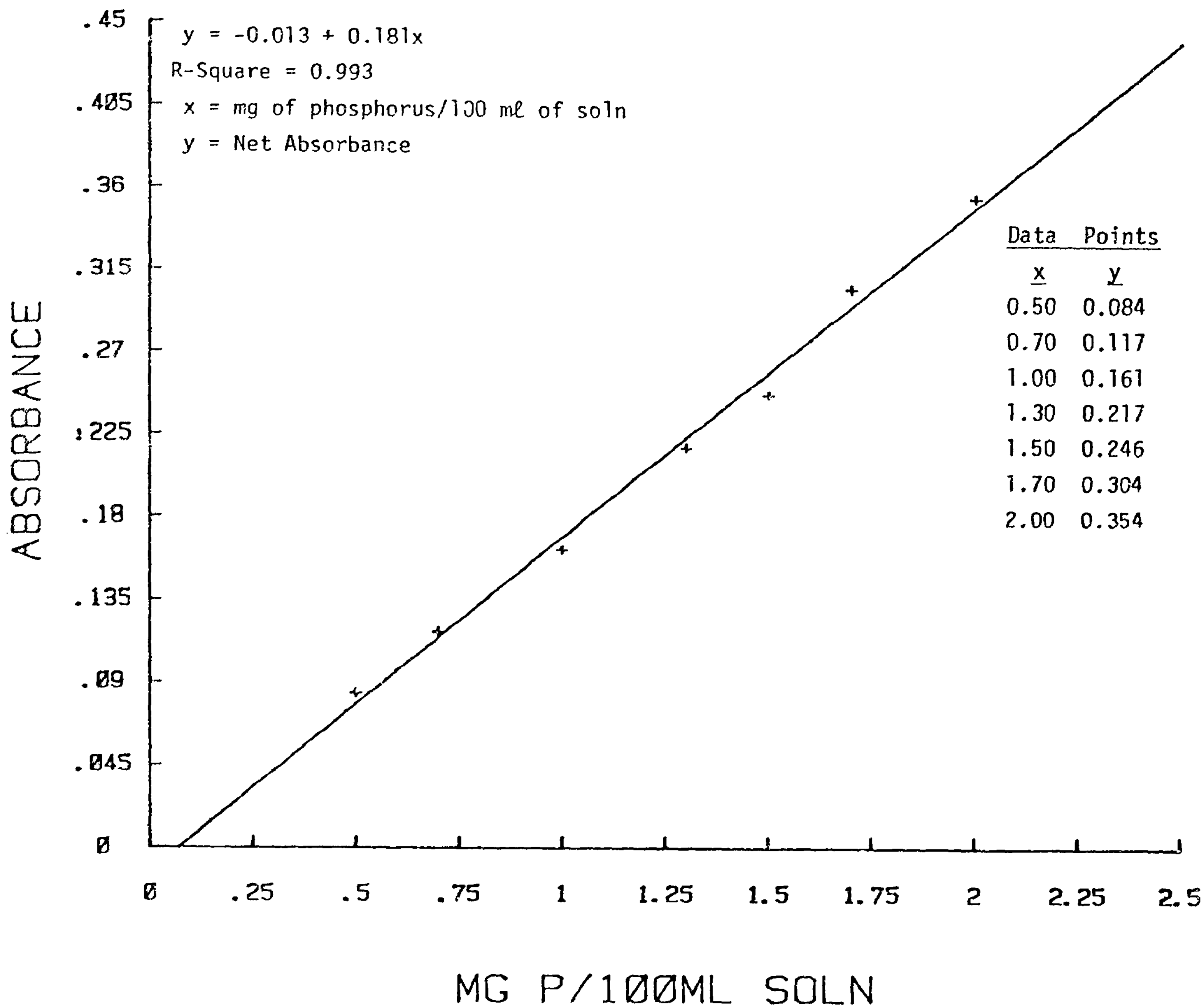


FIGURE 9. TYPICAL PHOSPHORUS CALIBRATION CURVE BASED ON A $Na_2HPO_3 5H_2O$ STANDARD

were within ±0.2 °C. The dew point hygrometer was calibrated with standard humidity environments established with saturated salt solutions. The relative humidity measured by the dew point probe was within ±4 percent of the standard value (Table 3).

The performance of the dew point hygrometer was checked with a wet and dry bulb psychrometer. The performance of the hygrometer is acceptable if the RH is within ± 6 percent from the psychrometer value.

E. Oxygen

Oxygen concentration in the chamber atmosphere was monitored with a commercial oxygen analyzer. An integral pump draws gas through the instrument at a pre-determined rate and oxygen in the gas stream is sensed by a solid state oxygen detector. Instrument readout is presented as percentage of oxygen in the sample gas stream with the range spanning 0 to 25 percent. Prior to use the analyzer must be allowed to "warm up" for 30 minutes to stabilize.

The instrument calibration was verified against ambient laboratory air diluted with high purity nitrogen (Table 4). To perform the calibration, the rate at which gas was sampled by the instrument, (F2), and the rate at which nitrogen dilution gas was introduced into the mixing chamber from which the instrument was sampling, (F1) were determined. These flow rates were measured using Hasting bubble flow meters of appropriate size whose calibration was traceable to NBS standards. F2 was determined at 2161.9±0.7 percent ml/min. F1 which was varied, to obtain different oxygen levels, was in the range of 50 to 300 ml/min and was measured with an accuracy of at least 1 percent. The concentration of oxygen sampled by the instrument during calibration was calculated from the following expression:

$$\text{Percent } O_2 = \frac{F2 - F1}{F2} \, 20.95$$

where F1 = flow rate of dilution gas
F2 = flow rate of gas sampled
20.95 = percentage concentration of oxygen in ambient air

The overall accuracy of the calculated O_2 concentration was estimated at 1.5 percent thus these data indicate that the O_2 analyzer in the above range is accurate to at least 1.5 percent.

Table 3: STANDARDIZATION OF HYGROMETER

Saturated Solution	Standard Humidity Level, %	Hygrometer measurements	
		Temperature, °C	Relative Humidity, %
$CaCl_2$ @ 25°C	31	26.0	29.0
NaBr @ 25°C	58	27.5	54.0
$(NH_4)_2SO_4$ within 20-30°C	81	27.0	80.0

Table 4: CALIBRATION OF OXYGEN ANALYZER

O_2 Concentration, %	
Indicated on the Analyzer	Calculated in Sample Gas Stream
14.2	14.1
15.0	15.1
15.4	15.4
16.0	16.0
16.7	16.9
17.5	17.6
18.0	18.0
18.7	18.7
19.1	19.2
20.2	20.2

E. Analysis of Carbon Monoxide, Phosphine and Hexane

Grab samples of air inside the exposure chambers were obtained during RP/BR aerosol generation. Gas chromatography was used to analyze these samples for carbon monoxide, phosphine and hexane. The samples were obtained using 250 ml evacuated glass flasks sealed with glass/teflon high vacuum stopcocks containing a septum for sample removal with a gas syringe. The flasks immediately prior to use were evacuated on a laboratory gas handling vacuum line (mechanical oil pump and liquid nitrogen trap) to <1 mm Hg pressure. Pressure was measured on a Wallace and Tiernan pressure gauge (0-800 mm) graduated in millimeters. This pressure gauge was calibrated on a weekly basis, using the vapor pressure of n-pentane at 0° C, and was accurate to ± 3 percent. The glass flask was attached to the vacuum line using a high vacuum O-ring seal. A similar seal was used to attach a 1 ft length of 1/8 in OD stainless steel tubing to the flask when sampling the exposure chamber atmosphere through a suitable opening in the chamber wall. This tubing and connecting Swagelok had dead volume of less than 3 percent relative to the sample volume. All samples were obtained from the same nominal position in the chamber, midway between top and bottom and about 1 ft from the side wall. "Blank" samples were obtained from the air in the room containing the exposure chamber.

Carbon monoxide was measured with a Varian Trace Gas Analyzer fitted with a helium ionization detector. The stainless steel column 20 ft long and 2.0 mm ID was packed with molecular sieve 5A. The carrier gas, Matheson UHP Helium, was metered at 35 ml/min through the column, which was maintained isothermal at 100° C. The gas sample to be analyzed was introduced into the gas chromatograph via a gas sampling loop on the vacuum line. The latter was filled with a known pressure of gas from the sample flask attached to the gas handling line. The chromatograph was calibrated using a Matheson calibration standard containing 23.7 ppm(v) CO in dry air. Calibrations were made each day on which the chamber samples were analyzed. From these calibration data a minimum detectable limit for CO of 1 ppm(v) was estimated, with an overall accuracy of ± 15 percent for the reported analytical data.

Phosphine was measured on a Hewlett Packard 5840 gas chromatograph fitted with a nitrogen-phosphorus detector. A glass column 3 ft long and 2.0 mm ID was packed with porapak N, 100/120 mesh. The column was run using a temperature program of 75° C for 1.8 min, increasing temperature at 9° C/min to 190° C. The carrier gas used was zero grade helium at a flow of 25 ml/min. Chamber samples were injected into the chromatograph using a 5 ml gas tight syringe. The chromatograph was calibrated with an Air Products gas standard containing 13.0 ppm(v) phosphine in nitrogen. In generating data for the calibration curve microliter samples (20 μl maximum) of the calibration gas were injected into the chromatograph. A minimum detectable limit of 5 ppb(v) was estimated. Six chamber gas samples were analyzed. To increase the sensitivity of the analyses, 5 ml samples of gas were injected into the chromatograph. In addition, these analyses were usually made only a few minutes after collecting the sample to avoid possible loss of phosphine due to hydrolysis. No time dependence of the phosphine concentration was observed over a period of 30 min.

Hexane Grab samples of hexane were measured on a Hewlett Packard 5840 gas chromatograph fitted with a flame ionization detector. A glass column 6 ft long and 2.0 mm inner diameter was packed with chromosorb 102, 60/80 mesh. The column was run isothermally at 175°C with a helium carrier gas flow rate of 30 ml/min. Samples were injected into the chromatograph with a 1 mm gas tight syringe. The chromatograph was calibrated with a Matheson standard gas containing 0.907 percent hexane in nitrogen. A minimum detectable limit of 1 ppm(v) was estimated.

IV. AEROSOL HOMOGENEITY STUDIES

A. Approach

The objective of these studies was to evaluate spatial and temporal homogeneity of the chamber atmosphere in a three-dimensional array of points through a procedure of simultaneous sampling with animal surrogates in place. For the pilot chamber, sufficient numbers of sampling points were selected to allow for characterization of spatial aerosol homogeneity within the chamber along with a series of sequential samples that were taken from a single or from multiple randomly selected fixed points to define temporal homogeneity for a period corresponding to the duration of the longest exposure. The aerosols were monitored for mass concentration, particle size and total phosphorus content at three generator settings (aerosol concentrations) replicating all tests at each generator setting three times.

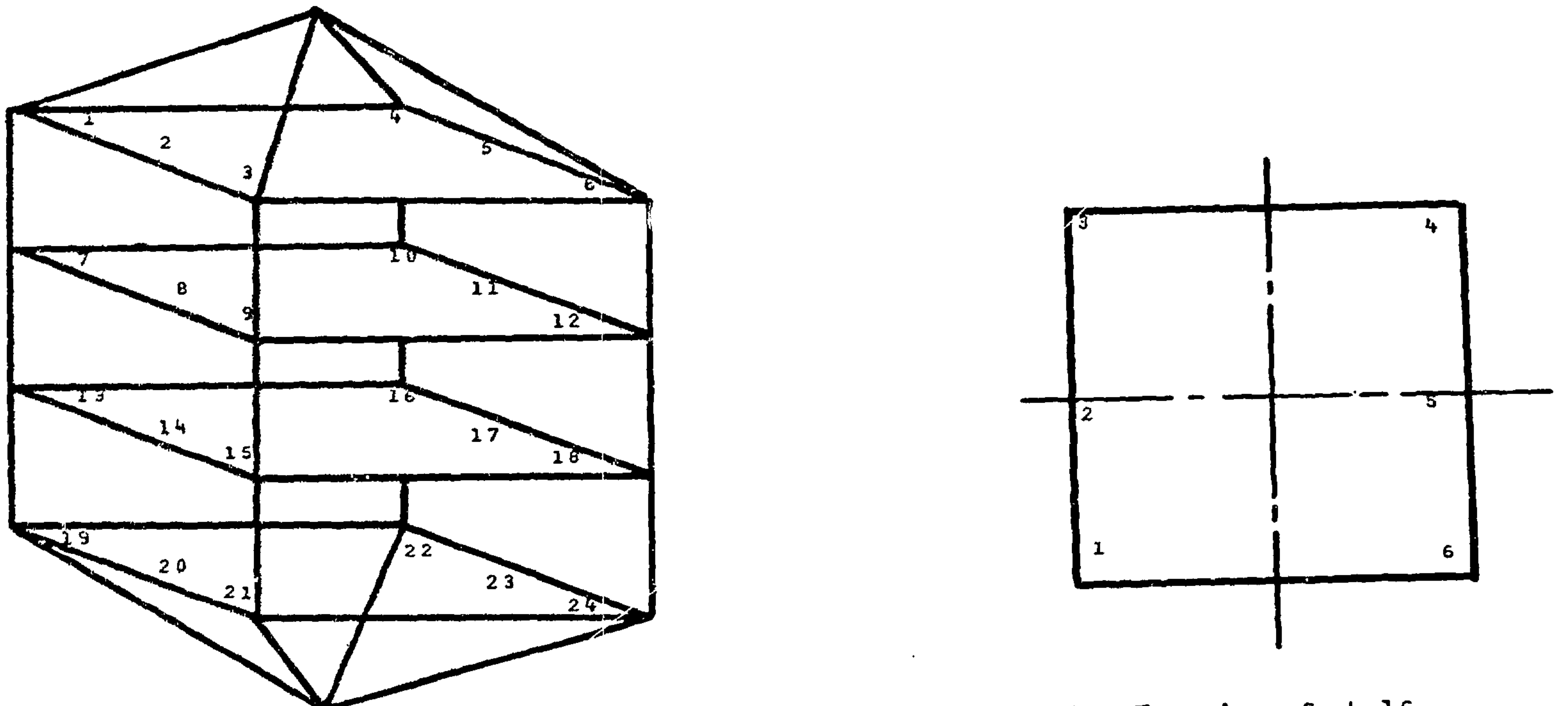
After standardization of the pilot chamber was completed, a single generator setting was randomly selected for each of the four remaining chambers and spatial and temporal homogeneity tests for the above mentioned aerosol parameters were conducted. Three replicate experiments were conducted for each generator setting i.e., for each chamber.

The ultimate objective was to reduce the variability of spatial and temporal homogeneity, with appropriate chamber modifications if necessary, to ± 20 percent of the mean of each parameter throughout the chambers and range of concentrations tested. This percentage limit was a change from the ± 15 percent originally targeted in the contract. The decision was based partly on experience obtained in exploratory aerosol generation experiments that demonstrated the limits in engineering controls of the generators in actual daily use associated with the variability in consistency of the RP/BR billets. In other words, aerosol concentrations were adjusted to specified levels using a generator extrusion pump setting established previously for that concentration. The observed extrusion pressures, and consequently, the extrusion rates and the resulting aerosol concentrations still varied, indicating possible differences in RP/BR billet consistency, or internal changes in the generator extrusion mechanics from burn to burn. In addition, although statistical analysis of the available pilot chamber data showed spatial and temporal homogeneity, it was not unusual for an occasional point to deviate as much as 20 to 30 percent from the overall chamber mean. In light of this and on the basis of the recommendation of IITRI's biostatistical consultant the spatial and temporal variability limits were revised from ±15 to ±20 percent of the mean.

1. Pilot Chamber

For the pilot chamber homogeneity studies a sampling schedule for 25 locations in a three-dimensional array of points (Figure 10, A and B) was designed based on aerosol physical considerations

In order to facilitate uniform access of the sampling probes for homogeneity testing into the chambers, an acrylic plastic panel was fitted to replace the front door for the duration of the homogeneity studies. Twenty-five 39 in long stainless steel tubes, were positioned so each shelf had three tubes set at each side for a total of six tubes per shelf plus one at the geometric center of the cubical portion of the chamber (Figure 11). The tubes at each shelf level entered at approximately the middle of the cage height and protruded into the chamber for a distance of 3, 18 and 33 in, respectively. This design assured that the aerosol samples always traveled the same distance from the sampling point to the collecting filters which were attached to the outside end of each tube. Aerosol samples for measurement of particle size also were taken from these locations. In addition a port at each of the groups of three tube locations plus one in the center of the chamber were



A. Schematic diagram of chamber

B. Top view of shelf

FIGURE 10: SAMPLING POINT LOCATIONS FOR AEROSOL HOMOGENEITY TESTS IN THE PILOT CHAMBER (No. 3)

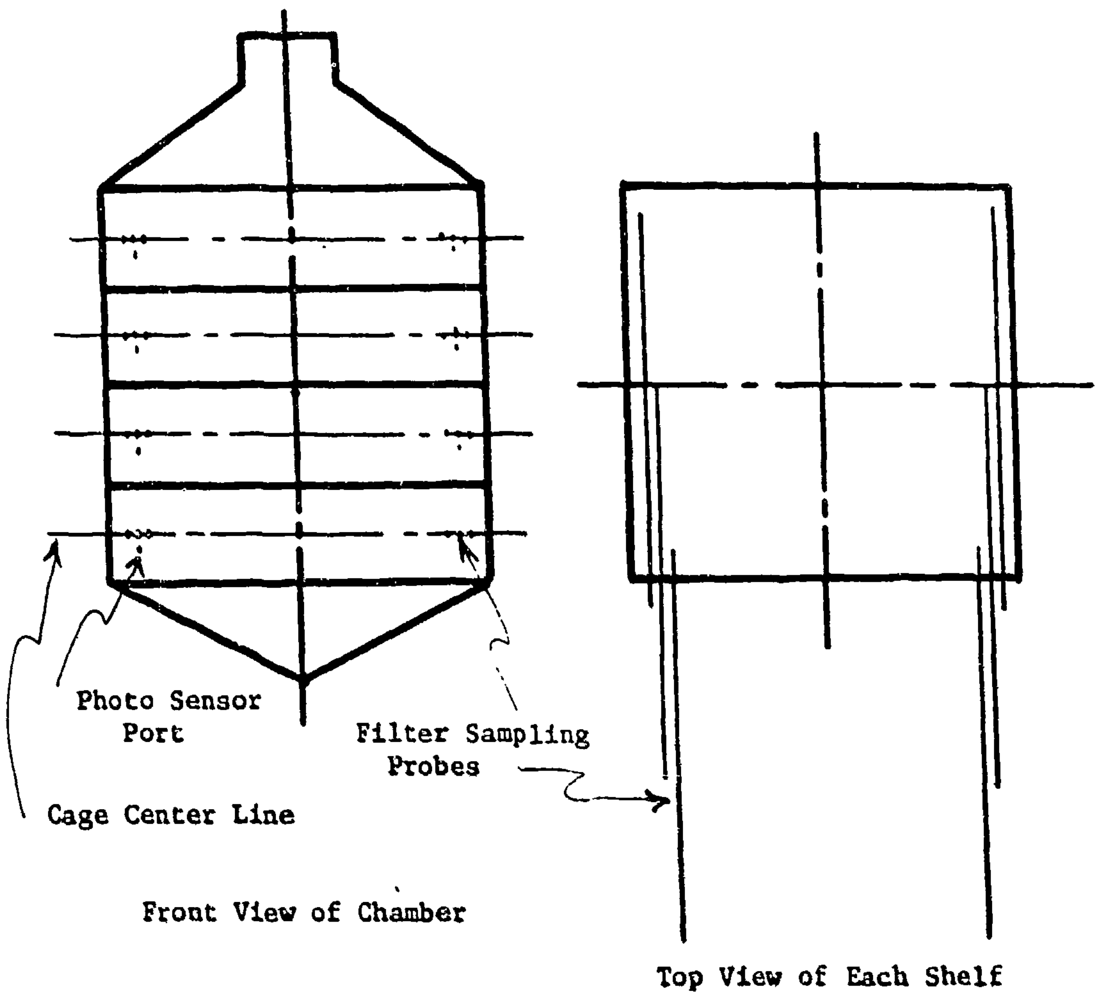


FIGURE 11. POSITIONING OF SAMPLING PROBES FOR AEROSOL HOMOGENEITY TESTING

provided for access for the optical sensors.

Aerosol homogeneity tests were conducted in the pilot chamber at 25 locations per concentration, at three concentrations with three replicate experiments at each concentration. The sampling methods used for determination of aerosol mass concentration and total phosphorus content by filter-collection, for aerosol mass concentrations with optical sensors, and for aerosol particle size by a QCM cascade impactor were previously described. (The mass mean aerodynamic diameter was used for testing spatial and temporal homogeneity of particle size). Similarly, measurements of temperature and relative humidity of the conditioned air and the oxygen levels in the exposure chambers have been also discussed. (Section III Chamber Sampling Methods). Temperature and relative humidity were monitored continuously and maintained in the specified ranges of 24-27°C and 40 to 60 percent RH. Oxygen concentration was measured in the chamber once during each replicate test and was consistently 21 percent.

The three test concentrations were selected on the following basis: the lowest operational concentration (C1: approximately 0.2-0.3 mg/l) of the RP/BR generators at the 500 liter/min constant air flow rates used in our chambers; the highest concentration (C3: approximately 1 mg/l) that could be maintained for the 4-hr testing periods using the larger 0.75 in diameter RP/BR billets provided by ORNL in the generators and operating at 500 liter/min flow rates; and an intermediate concentration (C2: approximately 0.5 mg/l) chosen between C1 and C3.

The concentrations were adjusted to these specified levels by using generator extrusion pump settings established in previous exploratory experiments. For each of the replicate tests, at a given concentration, the settings were maintained constant. The resulting extrusion pressures (and consequently the aerosol concentrations) still varied, indicating possible differences in RP/BR billet consistency, or internal changes in the generator extrusion mechanics from burn to burn. This could explain the residual variations in aerosol concentrations observed.

<u>Spatial homogeneity</u> of the aerosol was determined with filter-collected samples according to the outlines shown in Figures 12 A and B. Among the 25 sampling locations shown points were chosen in sets of five based on a stratified random sampling scheme. Each set consisted of four randomly selected points, one from each shelf and a common point (C or No 25) located at the center of the chamber (not on a shelf). In order to cover the entire 25 locations within the limits of available sampling instrumentation (sampling pumps and

gas meters) six sets of five simultaneous sampling points (set numbers 1-6, Figure 12B) were used for each replicate experiment at each aerosol concentration tested. For spatial homogeneity aerosol particle size was monitored sequentially at each of the 25 sampling locations with a QCM cascade impactor during each of the replicate experiments.

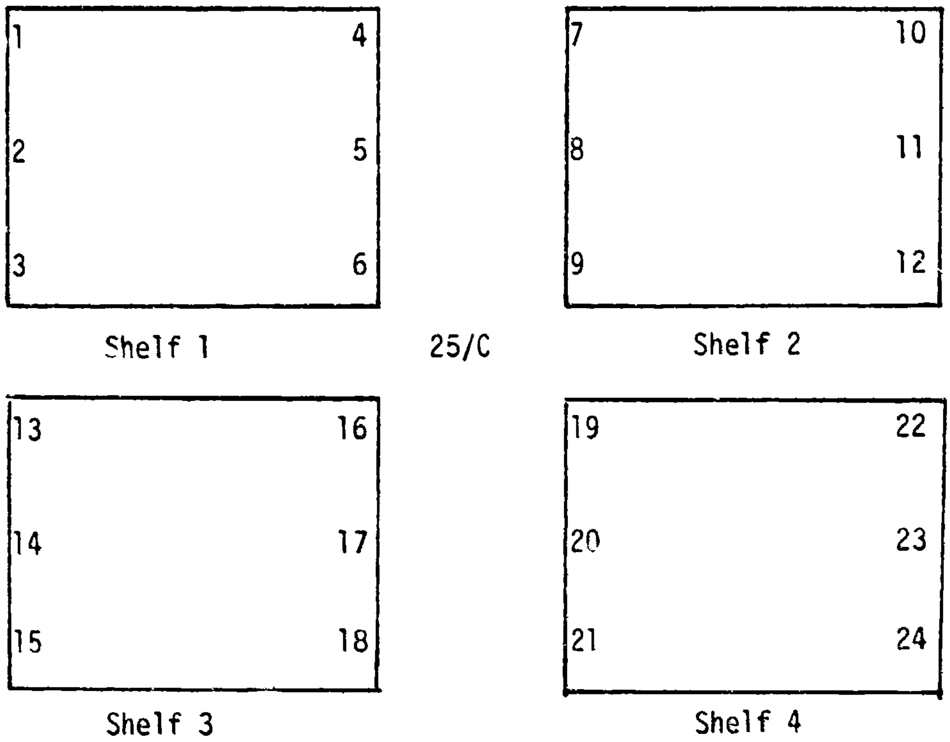
Temporal Homogeneity. For evaluation of temporal homogeneity four optical sensors were placed in fixed positions (set number 7, Figure 12B) randomly selected on the four shelves, with an additional sensor in the center of the chamber, position No. 25. Aerosol concentration at these locations was monitored and recorded continuously, for 4-hour periods, with the optical sensors connected to multiple strip chart recorders. To properly correlate the photosensor and the gravimetric aerosol mass concentration measurements integrated signal average readings were also taken simultaneously from the five photosensors at predetermined intervals during the continuous chart recordings. These integrated photosensor readings were used for statistical evaluation of temporal homogeneity. In addition, in position No.25 filter-collected sample-readings were also taken for determination of temporal homogeneity in coordination with the photosensor readings. Particle size temporal homogeneity was also determined in six sequential readings from this center position during a 4-hr period.

The randomization pattern was maintained constant for all three concentrations. Thus, the pilot chamber study produced 75 (3 concentrations x 25 locations) sets of filter-collected data for spatial homogeneity. For evaluation of temporal homogeneity there were 18 sets of data for filter- collected samples (3 concentrations x 1 location x 6 sampling periods) and 90 sets of data for the integrated photosensor readings (3 concentrations x 5 locations x 6 sampling periods). The sampling pattern for total phosphorus levels and particle size follows that of the filter-collected samples. A summary of the sampling frequencies for the spatial and temporal homogeneity tests is presented in Table 5.

2. Additional Chambers (Nos. 1, 2, 4 and 5)

Based on the statistical results obtained from the pilot chamber homogeneity test data the number of sampling points per chamber for Chamber Nos. 1, 2, 4 and 5 was reduced from 25 to 17. This modification was motivated by the results of statistical power calculations. These

A. Sampling Points on Chamber Shelves



B. Random Selection of Sampling Points

Shelf No.	Sampling Point Sets for: Filters 1	2	3	4	5	6	Photosensors 7
1	5	3	2	1	6	4	6
2	10	11	8	7	12	9	8
3	17	14	15	18	13	16	18
4	19	20	23	22	21	24	19
C	25	25	25	25	25	25	25

C not on shelf but in geometric center of chamber space

FIGURE 12. SAMPLING DESIGN FOR MEASUREMENT OF AEROSOL HOMOGENEITY IN PILOT CHAMBER (NO. 3)

Table 5: SAMPLING FREQUENCIES FOR SPATIAL AND TEMPORAL HOMOGENEITY TESTING IN THE PILOT CHAMBER[a]

Factors Affecting Sampling Frequency	Spatial Test Filter Samples	Temporal Test Photosensor	Temporal Test Filter Samples (No. 25)
Location	25	5 x 6[b]	1 x 6[b]
Concentration	3	3	3
Replication	3	3	3
Total Sample Number	225	270	54

[a] Aerosol mass concentration determined by optical sensor and gravimetric filter collection methods, (total weight and total phosphorus); aerosol particle size determined by a QCM.

[b] Six collection periods during the 4-hr periods for integrated photosensor readings and filter samples in Position No. 25.

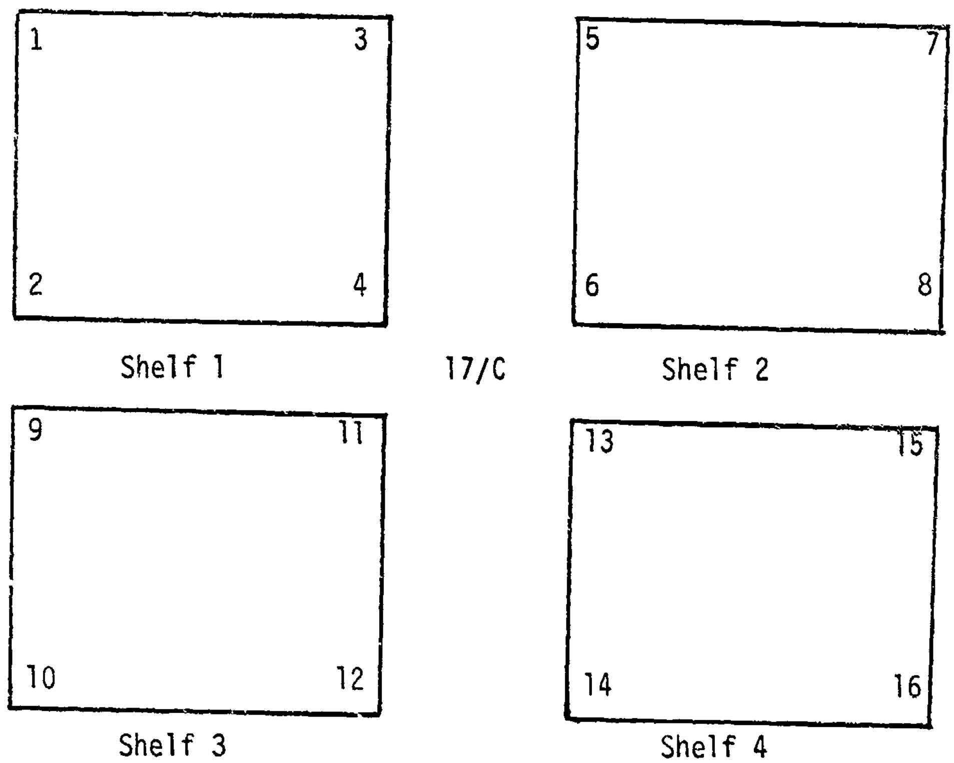
results indicated that with the relatively low variability observed in these data, 25 points produced extremely small type II (false negative) error rates, such that, deviations of only 5 to 10 percent were statistically significant. Therefore, the reduction of the number of sampling locations produced statistical sensitivity responsive to differences of 15 to 20 percent that was more concordant with the limitations of the aerosol generation and monitoring system and the variability in the RP/BR material. The revised sampling design is shown in Figures 13 A and B. Among the 17 sampling locations shown, points were chosen in four sets of five based on a stratified random sampling scheme. Each set consisted of four randomly selected points, one from each shelf and a common point (No. 17) located at the center of the chamber.

Spatial homogeneity of aerosol mass concentration was determined with filter collected samples. To cover the 17 locations four sets of five simultaneous sampling points were used (set numbers 1-4, Figure 13B) for each replicate experiment. Spatial homogeneity of particle size was monitored sequentially at each of the 17 sampling locations for each replicate experiment with the QCM cascade impactor.

Temporal homogeneity of aerosol mass concentration was evaluated by placing four photosensors in randomly selected fixed positions, one on each shelf (set number 7, Figure 13B) with a fifth photosensor in the center of the chamber (position No. 17). The aerosol concentration at these locations was monitored and recorded continuously for 4-hr periods with the photosensors connected to strip chart recorders. For comparison with the filter sample measurement and for statistical evaluation. Integrated signal average readings were taken simultaneously from the five photosensors at six predetermined intervals during the 4-hr test period. The center position (No. 17) was also sampled sequentially for aerosol concentration by filter samples and for particle size in coordination with the integrated photosensor readings during the 4-hr test period.

The above sampling schedule for Chamber Nos. 1, 2, 4 and 5 was further revised with respect to analysis of total phosphorus on the filter samples collected for gravimetric determination of aerosol mass concentration. This modification was also motivated by statistical power considerations. In the original design, the large number of samples would result in the rejection of the null hypothesis (i.e. rejection of spatial and temporal homogeneity) for deviations that are not meaningful for this analytical procedure. As

A. Sampling Points on Chamber Shelves



B. Random Selection of Sampling Points

Shelf No.	Sampling Point Sets for: Filters 1	2	3	4	5	6	Photosensors 7
1	3	4	2	1			4
2	5	6	8	7			6
3	10	9	11	12			11
4	15	14	16	13			16
C	17	17	17	17	17	17	17

C not on shelf but in geometric center of chamber space

FIGURE 13. SAMPLING DESIGN FOR MEASUREMENT OF AEROSOL HOMOGENEITY IN CHAMBER NOS. 1, 2, 4 AND 5

the studies progressed it became increasingly evident that in the total phosphorus determination the complexity of the analytical procedure was coupled with the aerosol filter-collection sampling. The potential experimental errors of both methods were carried over into the final analytical results of percent phosphorus in the chamber atmosphere thereby causing more variation in this parameter than originally expected from the precision estimated for the spectrophotometric method alone. In addition, because of the time consuming and labor intensive nature of the analytical method all other results in homogeneity testing were completed in each chamber long before there was any definitive information on the total phosphorus data. This made the final evaluation process rather cumbersome and caused delays in decision making. Thus after discussion with IITRI's consultant biostatistician, the sampling design was revised to include total phophorus analysis on four sampling positions located on shelf Nos. 1 and 4, (top and bottom) for a total of eight samples per replicate test. The determinations were conducted from the filters collected for mass concentration and the analysis was in accordance with the protocol specified for the Pilot Chamber tests.

B. Results of Statistical Analysis

The goal of the statistical analysis of those data was to test the null hypotheses of the spatial and temporal homogeneity in five inhalation chambers. In the presence of statistically significant within-chamber location differences the null hypothesis of spatial homogeneity must be rejected and it can be concluded that the chambers were spatially heterogeneous. In the presence of statistically significant between time-point differences the null hypothesis of temporal homogeneity must be rejected and it can be concluded that the chambers were temporally heterogeneous. In the absence of statistical significance, it could be concluded that the chambers were both spatially and temporally homogeneous. Furthermore, the consistency of homogeneity conditions across different concentrations and different chambers was also examined. Spatial and temporal homogeneity was tested in terms of aerosol mass concentration (by filter sampling, and from photosensor readings), particle size (mass mean aerodynamic diameter) and percent phosphoric acid (from filter collected samples).

A note of caution, as previously indicated due to the large number of chamber locations and replications, differences that are statistically significant may not be of any practical importance. In other words, the large number of samples would result in the rejection of spatial and temporal homogeneity for deviations that are not meaningful from an engineering perspective. This is due to the finding that the reproducibility of individual experiments was extremely high. This exceptional reproducibility (low variability between replicates) coupled with the large number of locations (large sample sizes), causes statistical significance to occur for extremely small between location deviations (5 to 10 percent). In order to obtain sensitivity at the a priori level of 15 to 20 percent, which is within the known limits of generator performance and variability in the RP/BR material, our only alternative was to decrease the sample size, that is sample fewer locations. In the following both the statistical significance of results as well as the effect size expressed in terms of percent mean deviations for locations and/or time points is described. The joint Type 1 (false positive) error rate was set a priori at 5 percent and an effect size of ±20 percent from the overall chamber mean was chosen.

1. Statistical Model

The statistical model used for the analysis of the Pilot Chamber (No.3) was a three factor mixed-model analysis of variance. Concentration (target concentrations of 0.2, 0.5 and 1.0 mg/l) and location (shelf Nos. 1, 2, 3, 4 and center point C) were considered to be the fixed factors in the design, whereas replication (1, 2 and 3) was considered random; hence the term "mixed model". This model determines if: a) between location differences are nonsignificant (there is spatial homogeneity) and if b) differences between locations depend on concentration (there is a concentration by location interaction).

Similarly, the analysis of temporal homogeneity also utilized the three-factor mixed-model analysis of variance. In this case, time was substituted for location as the second factor in the design. For the case of photosensor readings, temporal data were collected at five randomly selected locations; therefore, the spatial by temporal interaction (i.e. location by time) was also evaluated.

Between chamber comparisons were made by comparing overall means and examining deviations between the parameters measured in the pilot chamber (No. 3) and each of the other chambers (Nos. 1, 2, 4 and 5) at appropriate concentrations Finally, individual location levels were also reported in percent mean deviation units

$$\text{Percent Deviation} = \frac{X_{ij} - X_i}{X_i} \; 100$$

where i designates chamber number and j location.

2. Pilot Chamber (No. 3)

The means and standard deviations (SD) and the numbers of samples taken (N) at each sampling location and for each parameter tested are summarized in the Appendix in Table A-1 for the Pilot Chamber (No. 3) and Table A-2 for Chamber Nos. 1, 2, 4 and 5.

Spatial Homogeneity. Filter collected samples measuring aerosol mass concentration were found to be spatially homogeneous ($F=1.95$, $df=4/252$, $p=0.19$). This homogeneity was consistent for all three concentrations ($F=1.52$, $df=8/252$, $p=0.29$). Similarly, phosphoric acid levels determined on the filter samples were also found to be spatially homogeneous ($F=2.34$, $df=4/230$, $p=0.19$) and homogeneity was not affected by concentration ($F=0.37$, $df=8/230$, $p=0.69$). In contrast, particle sizes were not homogeneous ($F=18.01$, $df=4/189$, $p<.0001$). Particle sizes increased from the top to the bottom of the chambers and also increased with increasing concentrations. However the size of this shelf to shelf variability did not increase with concentration ($F=1.94$, $df=8/189$, $p=0.19$)

Temporal Homogeneity. Temporal homogeneity was measured in two ways. First, time averaged photosensor readings (measuring aerosol mass concentration) were determined at five locations across six time-points over four hours. Second, filter samples, (for aerosol concentration), particle size and phosphoric acid levels were repeatedly measured for six time intervals over a 4-hr period at the single center point. These two approaches allowed for more complete characterization of temporal homogeneity.

Aerosol concentration as determined by filter collected samples was temporally homogeneous as a whole ($F=0.63$, $df=5/34$, $p=0.59$), and for each concentration tested ($F=0.62$, $df=10/34$, $p=0.$[illegible]). Also in terms of photosensor measurements, aerosol concentration demonstrated temporal homogeneity ($F=.71$, $df=5/248$, $p=0.59$) which remained consistent at all concentrations tested ($F=1.71$, $df=10/248$, $p=0.14$). An important finding was that particle size remained temporally homogeneous ($F=0.62$, $df=5/34$, $p=0.59$) although statistically there was spatial heterogeniety(see above). Similarly, homogeneity was not affected by concentration (F=1.02, df=10/34, p=0.29). Finally, phosphoric acid levels also exhibited temporal homogeneity across all concentrations (F=0.47, df=5/34, p=0.69) and there was no concentration by time interaction (F=1.10, df=10/34, p=0.29).

3. Inter-Chamber Comparison

In an effort to verify that the temporal and spatial homogeneity obtained in the pilot chamber was consistent with that in the other four chambers, (Chamber Nos. 1, 2, 4 and 5) statistical analysis for each chamber was performed. In addition maximum location deviations in terms of worst case shelf means were calculated for each of these chambers relative to the overall chamber means of each of the chambers, (Table 6). For chamber No. 1, the worst shelf mean deviated 5 percent from the overall chamber mean for filter samples, 15 percent for particle size, 4 percent for photosensors and 6 percent for phosphoric acid. The worst case for chamber No. 2 was 13 percent for filter samples, 9 percent for particle size, 4 percent for photosensors, and 4 percent for phosphoric acid. Similarly, the worst deviation for chamber No. 4 was 14 percent for filter samples, 12 percent for particle size, 4 percent for photosensors, and 3 percent for phosphoric acid. Finally worst case results for chamber No. 5 were 17 percent for filter samples, 8 percent for particle size, 6 percent for photosensors, and 2 percent for phosphoric acid. Given that the worst case for all chambers was a 17 percent deviation from the overall chamber means, we concluded that all chambers exhibited both spatial and temporal homogeneity.

Statistical significance levels, F statistics and degrees of freedom for tests of Chamber Nos. 1, 2, 4 and 5 are displayed in Table 7. Many of these tests were significant; however as previously discussed, the sensitivity of the statistical evaluation is beyond what can be required with the given engineering limitations of the system. In light of this, statistically significant deviations that are in the worst case only 17 percent, thus under the 20 percent variation limit we have set as our goal, are still considered to represent adequate levels of homogeneity.

Percent deviation for individual locations are displayed in Tables 8 and 9. These percentage deviations have been derived from the mean observation

Table 6: PERCENT DEVIATION FOR THE WORST CASE SHELF MEAN IN CHAMBER NOS. 1, 2, 4 AND 5 FROM THE OVERALL CHAMBER MEAN

Chamber	Percent Deviation from Overall Chamber Means for Filters	Particle Size	Photosensor	% H_3PO_4
1	5	15	4	6
2	13	9	4	4
4	14	12	4	3
5	17	8	6	2

Table 7: STATISTICAL EVALUATION PARAMETERS FOR CHAMBER NOS. 1, 2, 4 AND 5

Chamber No.	Aerosol Mass Concentration: Filter Samples (all locations)			Aerosol Mass Concentration: Filter Samples (Center[17]location)			Aerosol Mass Concentration: Photosensor			Particle Size			% Phosphoric Acid		
	F	df	p<	F	df	p<	F	df	p<	F	df	p<	F	df	p<
1	3.7	4/53	.009	0.2	5/9	.97	3.0	5/54	.02	27.5	4/52	.0001	0.44	4/41	0.78
2	3.2	4/59	.02	0.9	5/10	.54	6.0	5/58	.0002	8.4	4/59	.0001	0.41	2/38	0.67
4	5.8	4/56	.0006	0.7	5/10	.65	0.5	5/58	.77	1.8	4/56	.14	0.45	2/21	0.65
5	7.2	4/58	.0001	0.6	5/10	.68	1.7	5/57	.14	5.5	4/58	.0009	0.91	2/21	0.42

Table 8. PERCENT DEVIATION OF MEANS OF INDIVIDUAL SAMPLING POINTS FROM OVERALL CHAMBER MEANS IN THE PILOT CHAMBER[a]

Sampling Position No.	Percent Deviations of Sampling Position Means from Overall Means in Pilot Chamber											
	Target Concentration 1 (0.2 mg/l)				Target Concentration 2 (0.5 mg/l)				Target Concentration 3 (1.0 mg/l)			
	Filter	Photosensor	Particle Size	H_3PO_4	Filter	Photosensor	Particle Size	H_3PO_4	Filter	Photosensor	Particle Size	H_3P
1	10.4		18.2	10.2	4.9		5.4	37.5	6.5		8.2	1.
2	0.0		7.1	4.4	5.4		6.2	0.4	6.5		13.2	
3	10.4		12.1	3.4	5.4		14.7	0.3	1.8		9.4	7.
4	4.1		4.5	25.7	8.1		18.6	6.6	4.6		12.0	4.
5	4.1		13.1	3.6	1.2		20.1	8.7	6.2		18.9	
6	9.4	13.0	22.7	28.6	7.1	3.6	17.4	0.9	7.7	6.0	2.5	1.
7	22.9		13.1	15.9	2.7		3.8	16.3	5.8		0.0	3.
8	12.5	15.8	6.0	0.2	10.3	5.4	1.5	4.9	0.9	7.9	1.3	0.
9	34.4		4.5	13.6	11.4		10.1	2.1	5.8		10.1	0.
10	13.5		6.1	2.7	8.7		22.5	22.4	7.1		7.5	0.
11	12.5		2.1	12.1	14.2		4.6	9.4	4.3		5.7	
12	21.8		19.2	12.8	6.5		9.3	0.4	14.8		3.1	11.
13	13.5		0.0	15.8	5.5		1.1	2.6	7.7		3.1	13.
14	25.0		9.1	7.7	3.3		7.7	6.5	3.1		10.7	
15	4.1		1.0	11.0	4.9		3.8	0.5	1.5		4.4	1.
16	13.5		3.0	3.1	3.3		10.8	1.7	6.8		6.3	0.
17	1.0		1.0	1.5	4.5		6.9	0.3	3.1		0.6	
18	5.2	4.8	10.1	3.9	4.9	14.8	0.8	20.9	5.6	11.6	8.2	7.
19	4.2	22.7	9.1	0.8	0.4	19.3	12.4	4.5	2.8	15.6	8.2	4.
20	8.3		7.5	1.7	7.1		16.2	4.8	0.0		14.5	
21	7.3		5.0	13.2	6.0		10.4	1.1	1.9		9.4	0.
22	13.5		7.6	13.1	4.9		6.9	9.2	8.0		10.0	4.
23	1.0		6.0	4.3	1.6		10.8	2.7	0.6		16.9	
24	30.2		12.0	2.0	13.6		14.7	11.2	0.3		12.0	0.
25[b]	3.5	5.5	0.0	2.4	1.2	5.6	9.2	3.6	0.4	1.1	0.6	2.

[a] The data were derived from 3 replicate experiments.

[b] Chamber center point.

Table 9. PERCENT DEVIATIONS OF MEANS OF INDIVIDUAL SAMPLING POINTS FROM OVERALL MEANS IN CHAMBER NOS. 1, 2, 4 AND 5 RESPECTIVELY.[a]

Sampling Position No.	Percent Deviations of Sampling Point Means from Overall Means in Chamber Nos. 1, 2, 4 or 5															
	Chamber No. 1/Conc. 3(1.0 mg/l)				Chamber No. 2/Conc. 2(0.5 mg/l)				Chamber No. 4/Conc.1(0.2 mg/l)				Chamber No. 5/Conc.3(1.0 mg/			
	Filter	Photo-sensor	Particle Size	H_3PO_4	Filter	Photo-sensor	Particle Size	H_3PO_4	Filter	Photo-sensor	Particle Size	H_3PO_4	Filter	Photo-sensor	Particle Size	H3P
1	7.6		18.5	2.9	11.7		11.1	2.3	2.5		8.3	2.7	18.6		20.2	3.
2	2.5		10.4	1.7	4.7		8.7	3.1	11.1		0.0	4.8	13.7		1.6	0.
3	6.9		23.1	3.1	16.3		3.9	5.1	6.2		17.7	0.4	18.6		19.1	1.
4	1.9	0.3	12.2	1.8	16.9	0.9	16.6	1.8	17.2	0.9	2.1	2.6	15.8	0.2	1.6	1.
5	6.6		8.3	5.6	16.3		7.1		20.3		1.0		14.3		3.8	
6	3.5	1.2	8.3	4.1	18.3	5.0	11.9		7.4		12.5		20.8	7.7	7.6	
7	3.5		2.5	2.8	3.2		1.6		9.9	15.2	3.1		15.8		5.4	
8	13.0		6.4		28.0		3.2		13.6		2.1		8.4		0.0	
9	8.6		1.9	9.9	18.3		6.3		17.3		1.0		15.2		3.8	
10	4.1		8.9		11.1		5.5		2.5		4.2		23.9		7.6	
11	10.2	0.1	8.9	8.4	7.2	3.6	10.3		4.9	4.6	4.2		9.0	0.5	4.9	
12	6.0		5.8	1.2	9.8		4.7		4.9		9.4	2.1	0.3		1.6	
13	12.4		8.7	1.7	14.4		6.4	18.1	16.1		23.4	2.6	20.2		1.6	2.
14	12.1		10.9	2.8	21.5		3.9	7.3	3.7		17.7	6.7	29.3		12.5	1.
15	3.1		13.4	3.3	15.6		23.0	6.2	11.1		2.1	3.3	8.4		7.1	2.
16	3.5	3.6	9.6	4.4	13.1	3.4	3.1	11.9	9.9	5.8	12.5	2.8	22.1	2.7	17.2	4.
17[b]	4.7	3.9	0.0	1.2	7.7	11.1	8.2	2.1	14.4	9.2	5.0		8.4	11.5	3.1	1.

[a] Each chamber was tested for all parameters at one of the concentrations previously tested in the pilot chamber. All data are derived from 3 replicate experiments. Target concentrations 1, 2 and 3 were 0.2, 0.5 and 1.0 mg/l respectively.

[b] Chamber center point.

data listed in the Tables A-1 and A-2 of the Appendix. For each parameter and at each test concentration the deviations for 25 sampling position means from the overall chamber means are summarized in Table 8 for the pilot chamber. Table 9 shows for Chamber Nos. 1, 2, 4 and 5, at one concentration each, the deviations of the means of 17 sampling positions from the respective overall chamber means.

Examination of these individual sampling location deviations throughout the five exposure chambers demonstrates that out of 431 locations shown in the tables a total of only 26 deviated more than 20 percent from the overall chamber means. This 6 percent figure is exactly what we would expect by chance alone. In addition, out of these 26 locations the four worst cases were in the pilot chamber (34 percent for filter samples, position No. 9, concentration C1 ; 23 percent for photosensors, Position No. 19, concentration C1 and 37 percent for phosphoric acid, Position No. 1, concentration C2) where spatial and temporal homogeneity have been previously established statistically. In fact, there were smaller deviations in the four additional chambers than in the pilot chamber. Thus these data show that the various parameters describing aerosol homogeneity stayed within the specified 20 percent limits of the overall chamber means 94 percent of the time for the five chambers; therefore, these observations support the conclusion that spatial and temporal homogeneity was achieved in all chambers.

In addition, inter-chamber comparisons were made. The overall mean for each parameter and for each chamber was compared to the overall means of the pilot chamber for that respective concentration level. The data in Table 10 demonstrate that all between-chamber comparisons were within 16 percent of the pilot chamber for all measured parameters; hence it was concluded that the targeted concentration values were attained in the additional chambers.

Again, statistical analysis of these data yielded mixed results. Chamber Nos. 2 and 4 were significantly different from their respective concentrations (determined by filter samples) in the pilot chamber (t_8=4.3; $p<0.01$ and t_8=5.1; $p<0.001$); however chamber Nos. 1 and 5 were not (t_8=1.1; $p<0.5$ and t_8=3.0; $p<0.8$). In contrast when concentrations determined by photosensors were compared to chambers No. 1 (t_{10}=7.69; $p<0.001$) and No. 5 (t_{10}=2.36; $p<0.05$) were different from the pilot chamber values, whereas chambers No. 2 (t_{10}=0.42; $p<0.7$) and No. 4

Table 10. COMPARISON OF OVERALL CHAMBER MEANS FOR AEROSOL MASS CONCENTRATION (FILTER COLLECTED AND PHOTOSENSOR DATA), PARTICLE SIZE AND PERCENT PHOSPHORIC ACID BETWEEN PILOT CHAMBER (NO. 3) AND CHAMBER NOS. 1, 2, 4 AND 5.[a]

Aerosol Characterized by:	Target Test Concentrations, mg/l: 0.2 (C_1)				0.5 (C_2)				1.0 (C_3)					
	Pilot Chamber No. 3		Chamber No. 4		Pilot Chamber No. 3		Chamber No. 2		Pilot Chamber No. 3		Chamber No. 1		Chamber No. 5	
	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD
Mass Conc, mg/l (filter sample)	0.32	0.09	0.27 (16%)	0.06	0.61	0.09	0.50 (15%)	0.09	1.08	0.11	1.04 (3%)	0.10	1.06 (2%)	0.17
Mass Conc, mg/l (photosensor)	0.23	0.07	0.22 (4%)	0.04	0.53	0.09	0.52 (4%)	0.05	0.99	0.11	1.06 (8%)	0.08	1.02 (4%)	0.14
Particle Size, μm	0.33	0.06	0.31 (3%)	0.07	0.42	0.08	0.42 (2%)	0.05	0.53	0.08	0.51 (4%)	0.06	0.61 (15%)	0.07
H_3PO_4, %	62.0	11.2	63.2 (2%)	7.4	60.3	10.2	54.9 (9%)	8.0	63.1	6.1	70.6 (12%)	7.4	71.5 (13%)	3.3

[a] Numbers in parenthesis indicate the percentage deviations of the means in Chamber Nos. 1, 2, 4 and 5 from the means determined in the Pilot Chamber at each target concentration.

(t_{10}=1.58; $p<0.2$) were not. In terms of particle size only chamber No. 5 was different from the pilot chamber (t_8=2.86; $p<0.05$) whereas chamber No. 1 (t_8=0.63; $p<0.7$); No. 2 (t_8=0.99; $p<0.6$) and No. 4 (t_8=0.52; $p<0.8$) were not. In case of phosphoric acid chambers No. 1 (t_8=6.04; $p<0.001$) No. 2 (t_6=3.83; $p < 0.01$) and No. 5 (t_6=9.17; $p < 0.001$ were different and No. 4 (t_6=0.91; $p<0.6$) was not.

The individual shelf and center point means as well as overall means obtained from multiple determinations of aerosol mass concentration particle size and percent phosphoric acid for each chamber and each concentration level tested are summarized in Tables 11, 12 and 13. Also shown in Table 14 are aerosol concentration means obtained from the five photosensor locations over the predetermined six time periods for each chamber and at each concentration. Comparing the overall means in Tables 11 and 14 shows generally good consistency for aerosol mass concentration determined by filter-collected samples and photosensor readings respectively. The correlation between the two methods improved after the measurements in the pilot chamber were completed and studies in the additional chambers were initiated because that was when the detailed calibration instructions for the photosensors were received and further improvements in the standardization procedure could be made.

It was mentioned before and it is also evident from Table 12 that particle size, expressed as mass mean aerodynamic diameter, appears to increase from top to bottom in each chamber (with aerosol residence time) and also with increasing aerosol concentration. The chamber gradient within each concentration, however is generally within the precision of the cascade impactor. On the other hand, the change in particle size for the entire concentration range tested was 0.3 to 0.6 um mass mean aerodynamic diameter which represents particles that can be inhaled and deposited in the deep lung.

When viewing the range in percent phosphoric acid data (Table 13), which was shown to be within the set variability limits of 20 percent, it must be emphasized that the spectophotometric determination involves a complex multistep analytical procedure that may introduce experimental errors after the actual "chamber monitored" measurements of the filter weights are taken.

Table 11: COMPARISON OF SHELF MEANS BETWEEN PILOT CHAMBER AND CHAMBER NOS. 1, 2, 4 AND 5 FOR AEROSOL MASS CONCENTRATION DETERMINED BY FILTER SAMPLES

Shelf No.	Pilot Chamber (No. 3) Conc. 1(0.2 mg/1) Mean	± SD	Pilot Chamber (No. 3) Conc. 2(0.5 mg/1) Mean	± SD	Pilot Chamber (No. 3) Conc. 3(1.0 mg/1) Mean	± SD	Chamber No. 1 Conc. 3(1.0 mg/1) Mean	± SD	Chamber No. 2 Conc. 2(0.5 mg/1) Mean	± SD	Chamber No. 4 Conc. 1(0.2 mg/1) Mean	± SD	Chamber No. 5 Conc. 3(1.0 mg/1) Mean	± SD
1	0.32	0.09	0.62	0.10	1.13	0.08	1.10	0.07	0.57	0.05	0.29	0.06	1.24	0.03
2	0.32	0.15	0.59	0.08	1.01	0.06	1.03	0.11	0.48	0.11	0.27	0.05	1.07	0.17
3	0.31	0.07	0.61	0.17	1.09	0.07	1.09	0.10	0.52	0.07	0.28	0.04	1.07	0.17
4	0.32	0.09	0.60	0.06	1.09	0.12	1.03	0.11	0.50	0.10	0.28	0.06	0.96	0.22
C[a]	0.31	0.05	0.62	0.08	1.08	0.13	1.00	0.09	0.47	0.08	0.23	0.05	0.99	0.06
Overall Mean	0.32		0.61		1.08		1.04		0.50		0.27		1.06	

[a] Chamber center point.

TABLE 12: COMPARISON OF SHELF MEANS FOR PARTICLE SIZE BETWEEN PILOT CHAMBER AND CHAMBERS NOS. 1, 2, 4 AND 5

Shelf No.	Pilot Chamber (No. 3) Conc. 1(0.2 mg/1) Mean	± SD	Pilot Chamber (No. 3) Conc 2(0.5 mg/1) Mean	± SD	Pilot Chamber (No. 3) Conc. 3(1.0 mg/1) Mean	± SD	Chamber No. 1 Conc 3(1.0 mg/1) Mean	± SD	Chamber No. 2 Conc. 2(0.5 mg/1) Mean	± SD	Chamber No. 4 Conc. 1(0.2 mg/1) Mean	± SD	Chamber No. 5 Conc. 3(1.0 mg/1) Mean	± SD
1	0.29	0.04	0.37	0.08	0.47	0.07	0.44	0.04	0.37	0.04	0.30	0.05	0.56	0.08
2	0.34	0.08	0.41	0.06	0.51	0.06	0.48	0.03	0.39	0.04	0.31	0.04	0.58	0.05
3	0.33	0.06	0.45	0.05	0.56	0.07	0.55	0.04	0.43	0.04	0.33	0.06	0.62	0.07
4	0.35	0.05	0.48	0.04	0.59	0.06	0.57	0.04	0.45	0.05	0.36	0.05	0.66	0.06
C[a]	0.33	0.16	0.56	0.16	0.53	0.08	0.52	0.05	0.45	0.04	0.30	0.09	0.63	0.05
Overall Mean	0.33		0.42		0.53		0.51		0.42		0.31		0.61	

[a] Chamber center point.

Table 13: COMPARISON OF SHELF MEANS FOR PERCENT PHOSPHORIC ACID IN AEROSOL BETWEEN PILOT CHAMBER AND CHAMBER NOS , 2, 4 AND 5

Shelf No.	Pilot Chamber (No. 3) Conc. 1 (0.2 mg/l) Mean	± SD	Conc. 2 (0.5 mg/l) Mean	± SD	Conc. 3 (1.0 mg/l) Mean	± SD	Chamber No. 1 Conc. 3 (1.0 mg/l) Mean	± SD	Chamber No. 2 Conc. 2 (0.5 mg/l) Mean	± SD	Chamber No. 4 Conc. 1 (0.2 mg/l) Mean	± SD	Chamber No. Conc. 3 (1.0 mg/ Mean	± S
1	63.8	15.66	54.3	11.08	65.07	4.83	71.70	6.19	55.13	8.33	64.75	6.43	72.37	2.1
2	58.9	13.85	58.3	14.64	61.03	4.70	70.93	3.74						
3	64.4	9.15	62.1	8.18	62.52	10.39	75.79	3.31			61.88			
4	59.4	7.92	58.7	8.45	61.65	5.28	69.61	5.79	56.35	7.91	61.80	8.53	70.53	4.22
C[a]	63.4	6.61	58.1	5.97	64.52	4.18	69.74	10.04	53.63	8.11			70.67	
Overall Mean	62.0		60.3		63.1		70.6		54.9		63.2		71.5	

[a] Chamber center point.

TABLE 14: COMPARISON OF PHOTOSENSOR TIME PERIOD MEANS BETWEEN PILOT CHAMBER AND CHAMBER NOS. 1, 2, 4 AND 5 FOR AEROSOL MASS CONCENTRATION

Time Period	Pilot Chamber (No. 3) Conc. 1 (0.2 mg/l)		Pilot Chamber (No. 3) Conc. 2 (0.5 mg/l)		Pilot Chamber (No. 3) Conc. 3 (1.0 mg/l)		Chamber No. 1 Conc. 3 (1.0 mg/l)		Chamber No. 2 Conc. 2 (0.5 mg/l)		Chamber No. 4 Conc. 1 (0.2 mg/l)		Chamber No. Conc. 3 (1.0 mg	
	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± S
1	0.24	0.07	0.52	0.09	0.98	0.12	1.10	0.10	0.54	0.04	0.23	0.04	1.04	0.2
2	0.30	0.04	0.50	0.12	0.95	0.08	1.07	0.07	0.54	0.05	0.22	0.03	0.96	0.1
3	0.25	0.10	0.54	0.08	0.98	0.10	1.07	0.07	0.50	0.03	0.23	0.04	0.98	0.1
4	0.23	0.04	0.54	0.09	0.98	0.11	1.06	0.08	0.52	0.05	0.23	0.04	1.02	0.0
5	0.20	0.05	0.52	0.07	1.00	0.12	1.06	0.06	0.57	0.04	0.23	0.03	1.05	0.1
6	0.18	0.04	0.53	0.10	0.96	0.11	1.03	0.05	0.51	0.24	0.22	0.04	1.08	0.1
Overall Mean	0.23		0.53		0.99		1.06		0.52		0.22		1.02	

C. Analyses for Carbon Monoxide, Phosphine and Hexane

1. Carbon Monoxide

On selected occasions during the homogeneity studies chamber air samples collected at representative RP/BR aerosol mass concentrations were obtained and analyzed for carbon monoxide. The data so obtained are presented in Table 15. For completeness, the calibration curves used in deriving the data are shown in Figure 14 and 15. With the exception of Study No. 35, the carbon monoxide concentration levels found in a given sampling were consistent with each other, although considerable variability from experiment to experiment was observed.

Of the data in Table 15, only those obtained on 2-4-83 and 2-23-83 showed any detectable carbon monoxide in the "blank" room air samples. Indeed these "blanks" appeared to contain more carbon monoxide than the chambers themselves. Such a finding did not seem reasonable and in most of the subsequent experiments "blank" air samples from the hallway outside of the laboratory were also analyzed as a possible check of the buildings carbon monoxide levels. In none of these samples was carbon monoxide ever detected. On balance it is believed that the data obtained in the experiments on 2-4-83 and 2-23-83 should be rejected.

From the remaining data, chamber carbon monoxide levels appear to vary between about 5 to 22 ppm(v) with no obvious correlation with chamber RP/BR aerosol loading. A similar variability in carbon monoxide levels was found in a previous study conducted in our laboratories on the composition of white phosphorus/felt wedge smoke.

2. Phosphine

A total of six exposure chamber air samples obtained at representative RP/BR aerosol test concentrations were obtained and analyzed. To increase the sensitivity of the analyses, 5 ml samples of the gas were injected into the chromatograph. A typical calibration curve is shown in Figure 16 and analytical data obtained are presented in Table 16. In only three of the chamber samples was phosphine detected. The concentration was so low that accurate quantification by the chromatograph peak area integrator was not possible. The numerical values listed in Table 16 are approximate upper limit values based on peak height measurements.

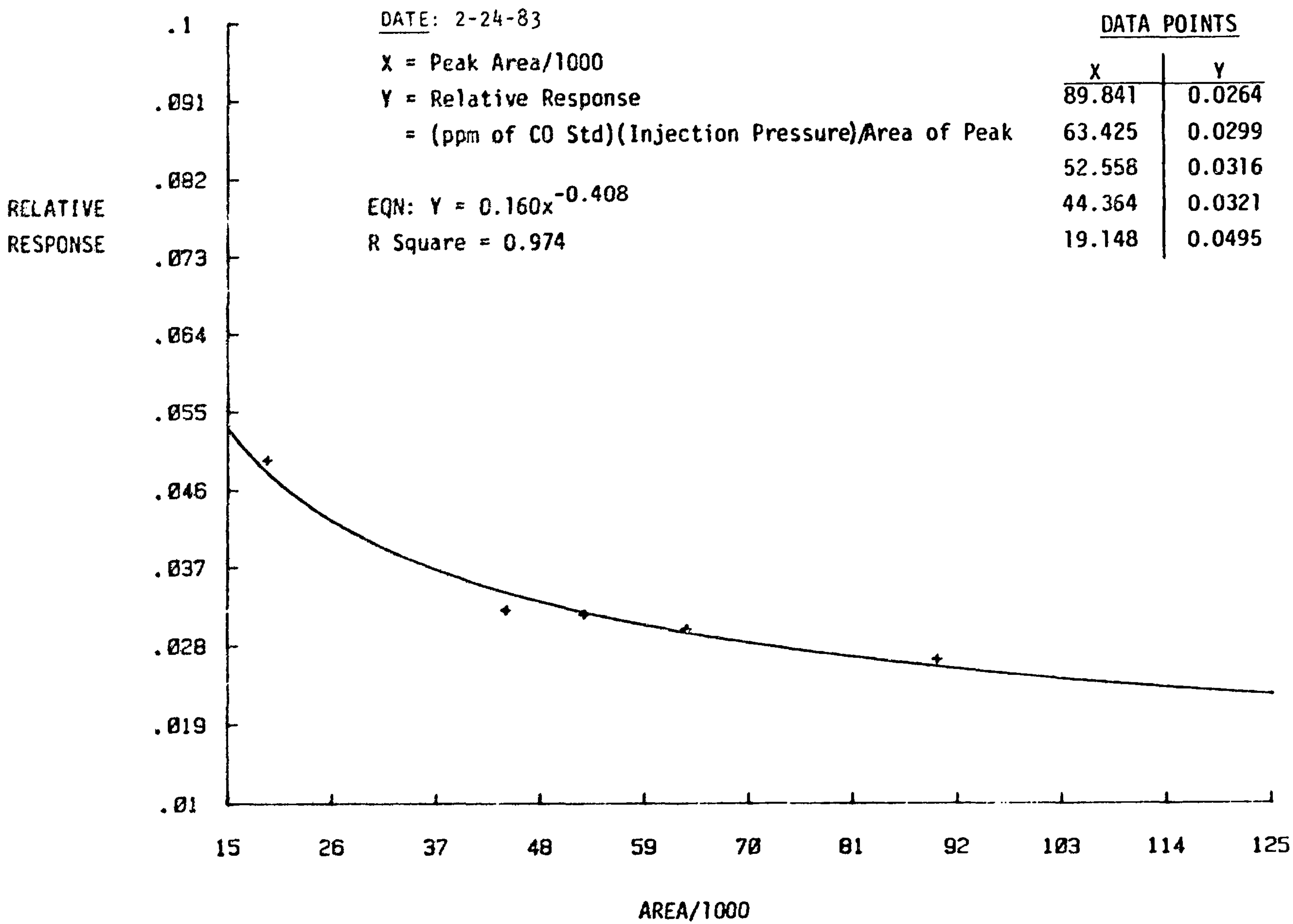
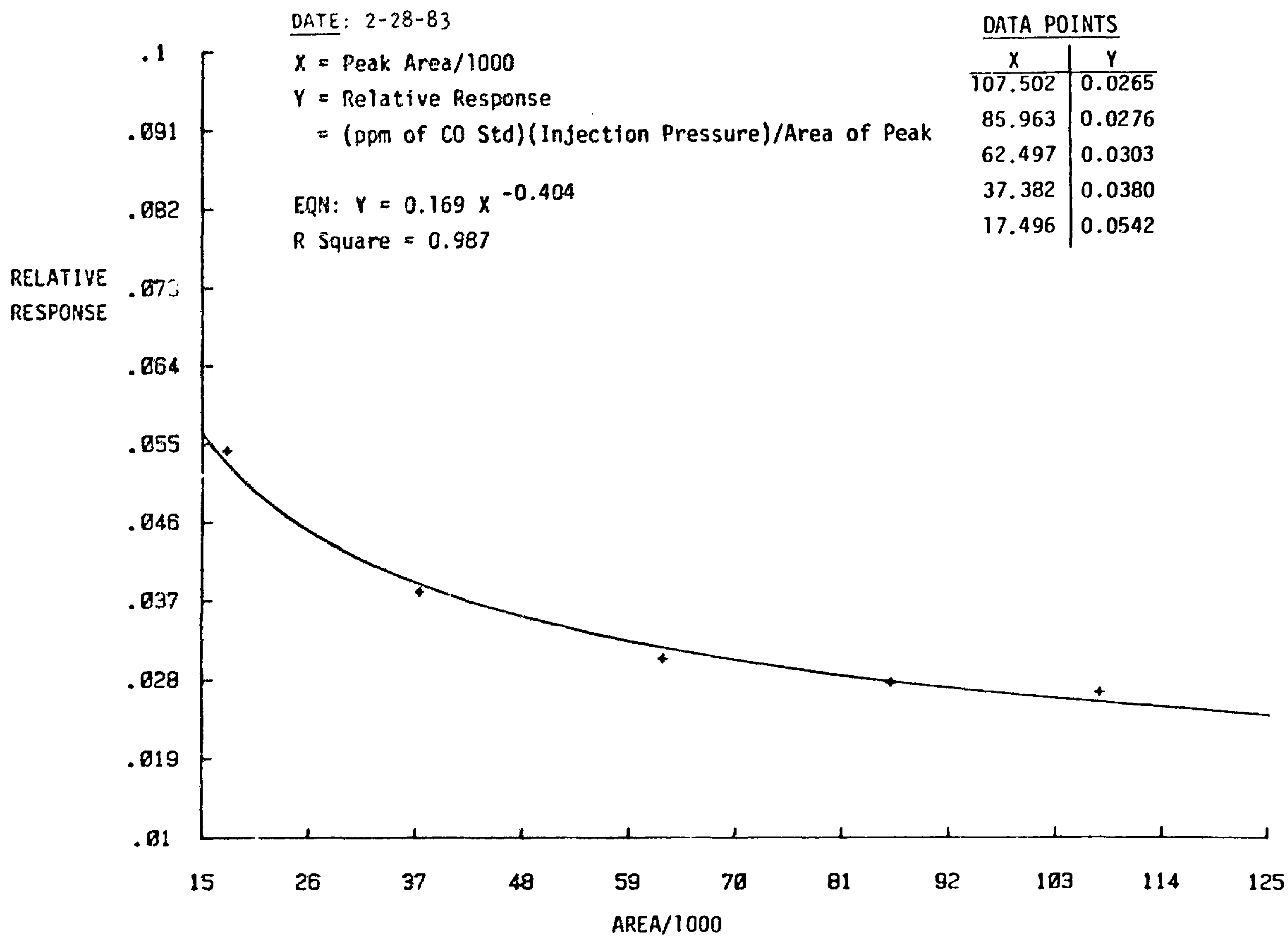


FIGURE 14: CARBON MONOXIDE CALIBRATION CURVES



X	Y
107.502	0.0265
85.963	0.0276
62.497	0.0303
37.382	0.0380
17.496	0.0542

FIGURE 14: CARBON MONOXIDE CALIBRATION CURVES

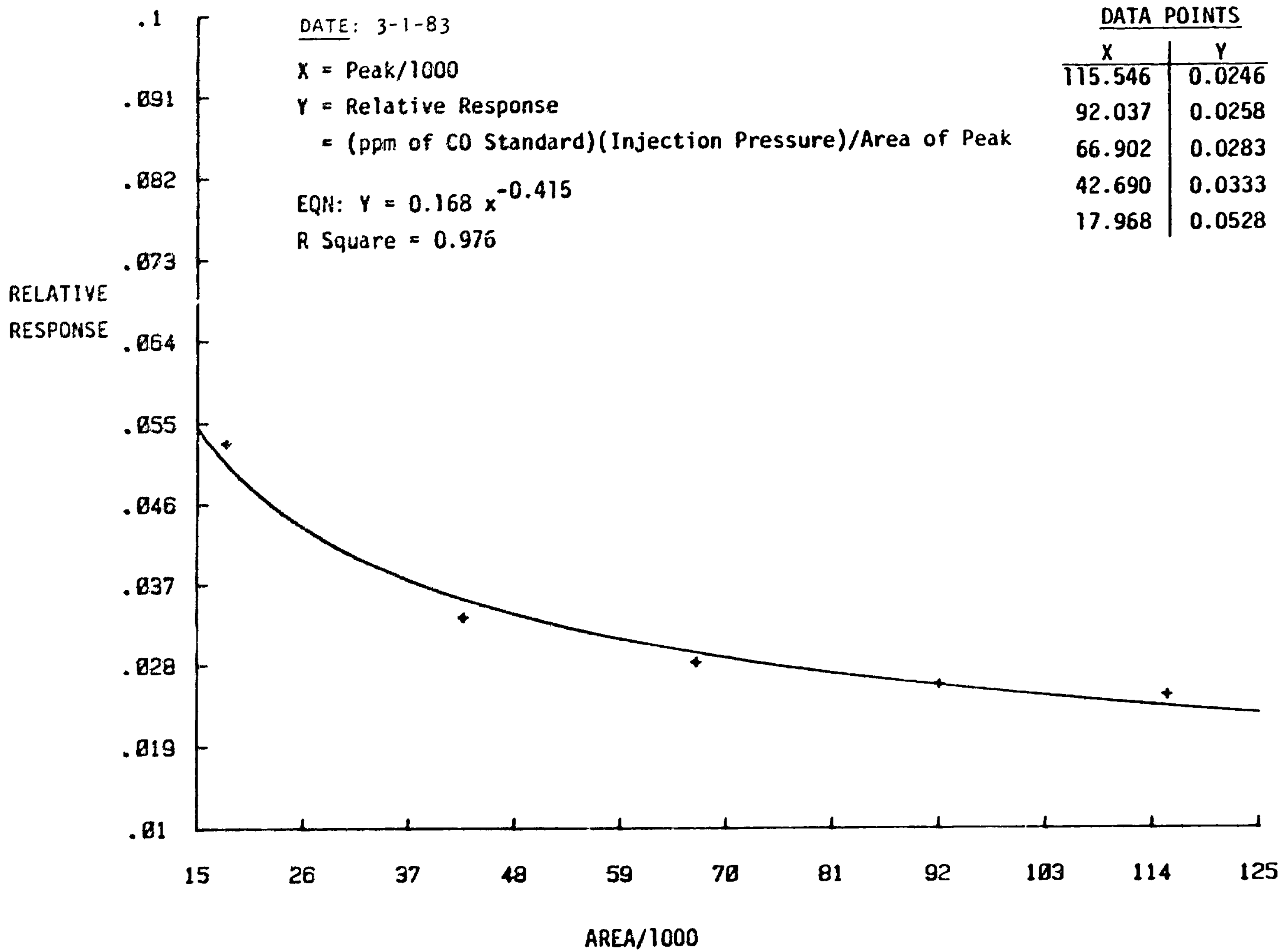


FIGURE 14: CARBON MONOXIDE CALIBRATION CURVES

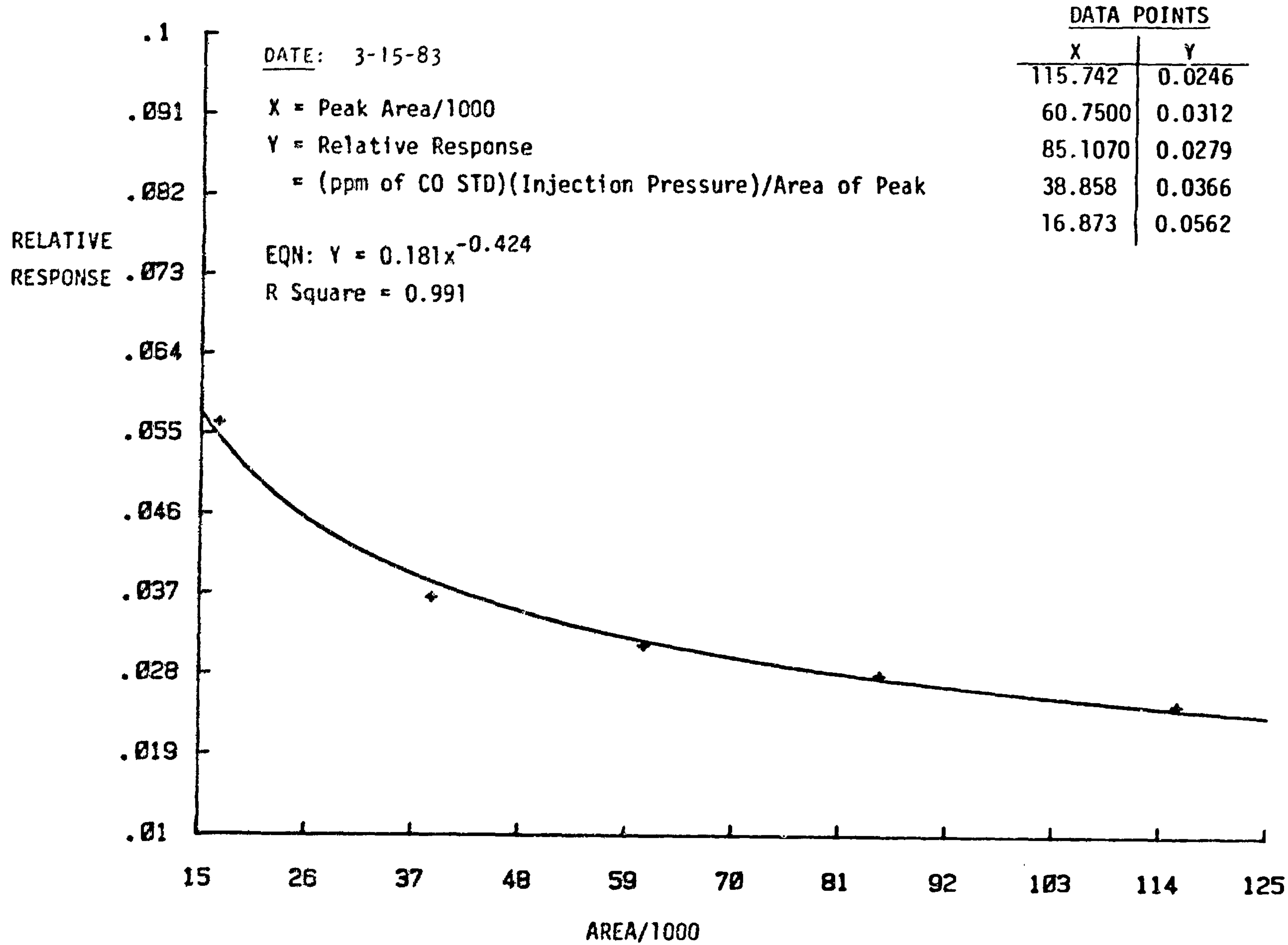
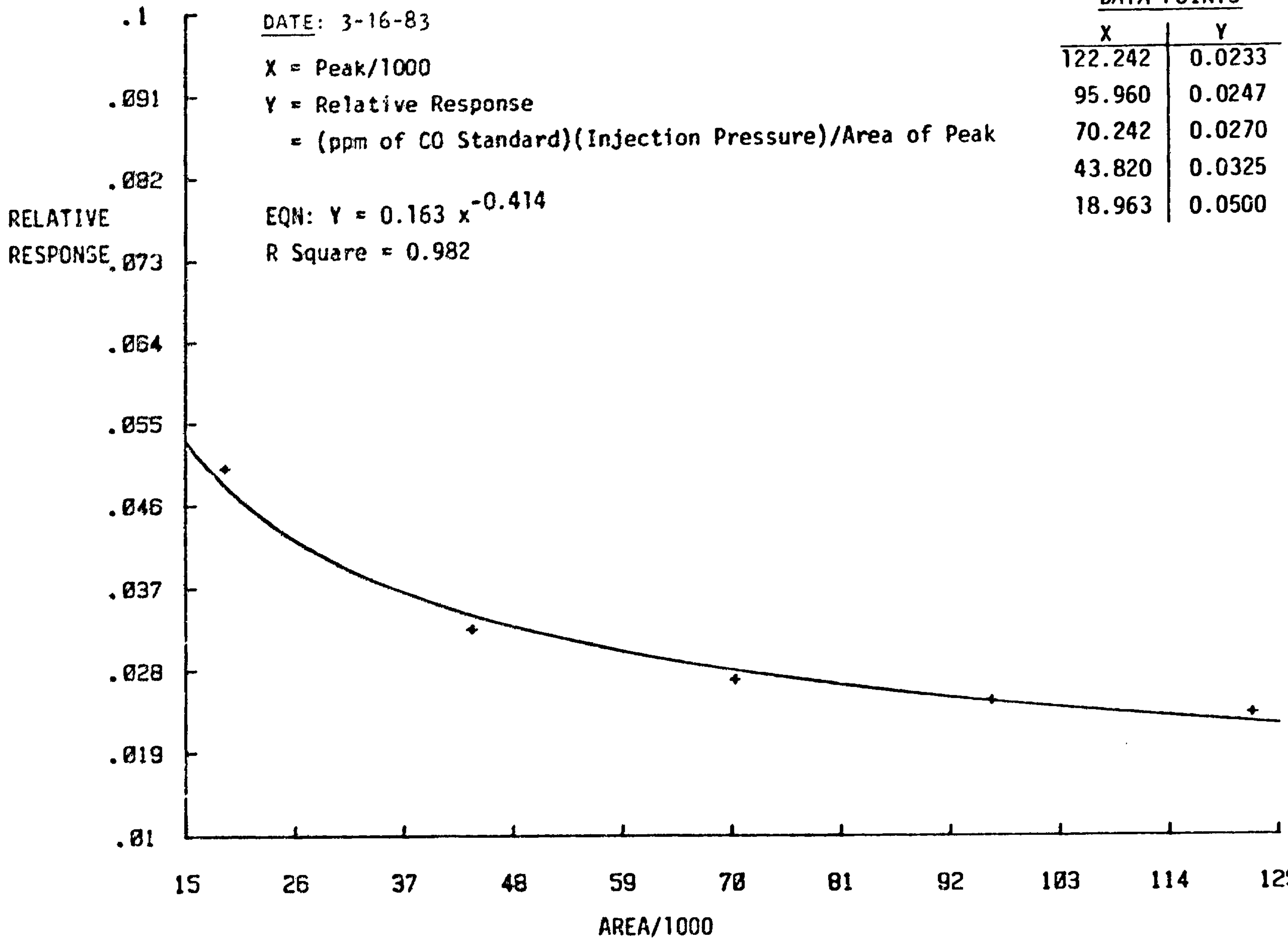


FIGURE 14: CARBON MONOXIDE CALIBRATION CURVES



DATA POINTS

X	Y
122.242	0.0233
95.960	0.0247
70.242	0.0270
43.820	0.0325
18.963	0.0500

FIGURE 15: CARBON MONOXIDE CALIBRATION CURVES

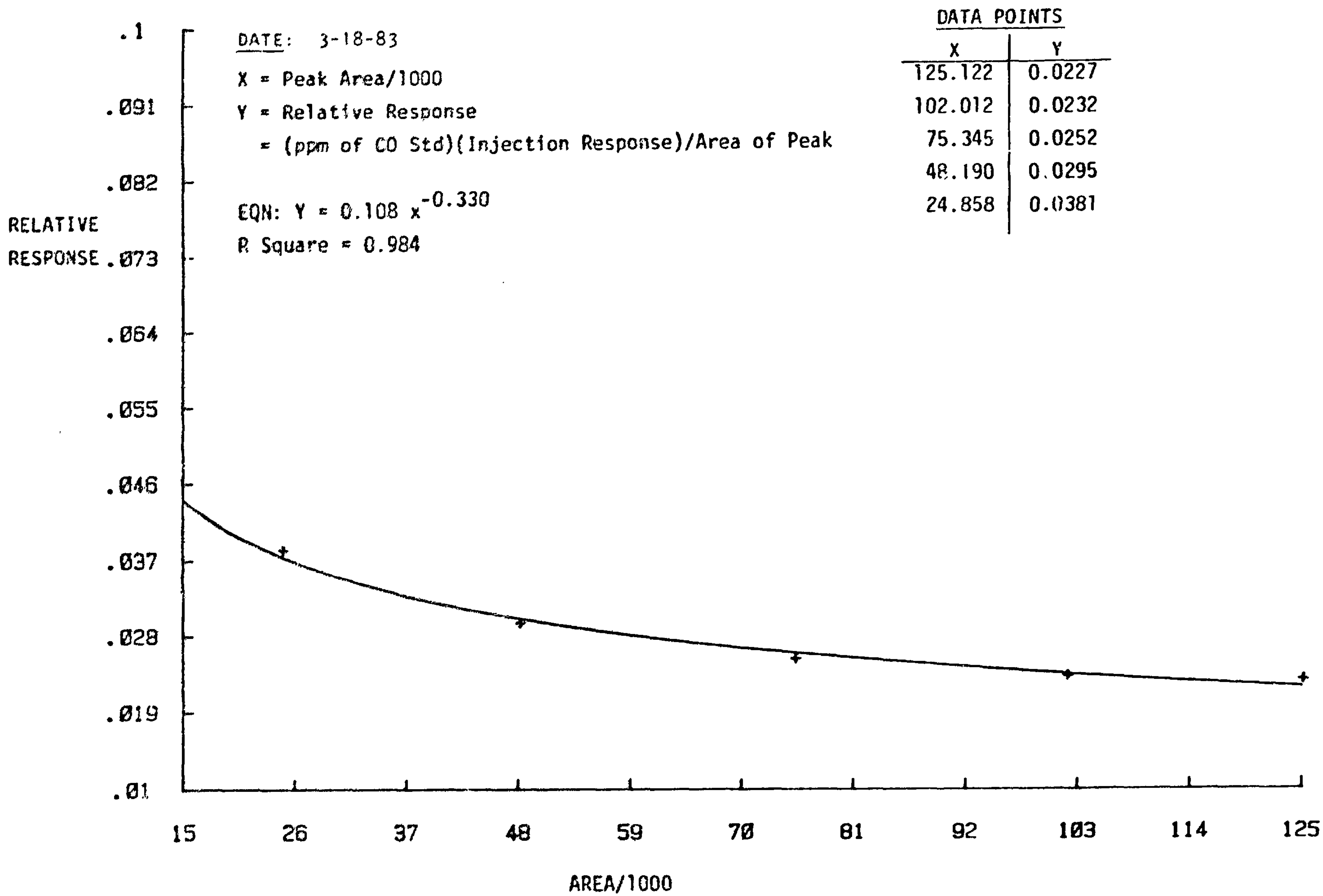


FIGURE 15: CARBON MONOXIDE CALIBRATION CURVES

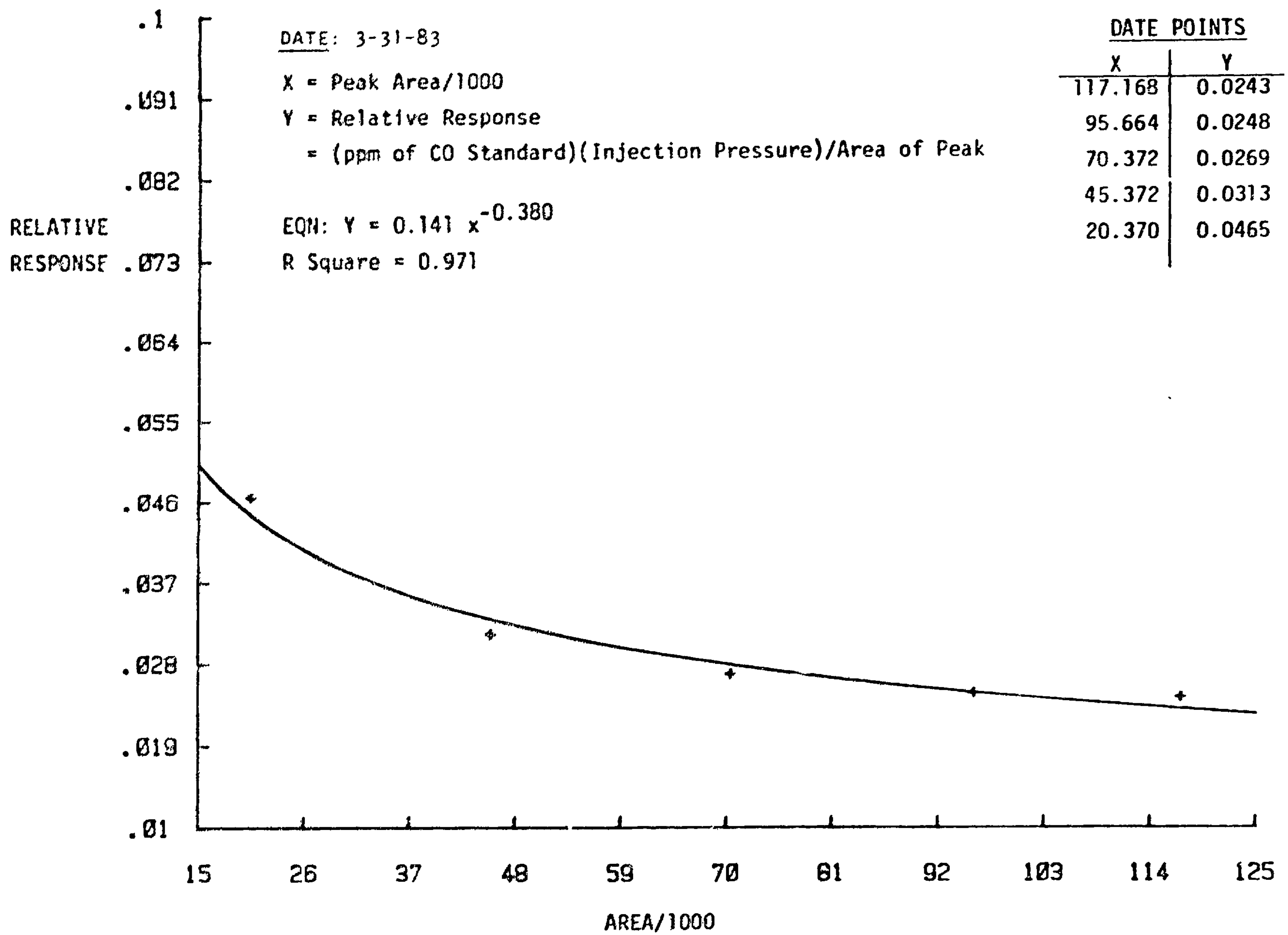


FIGURE 15: CARBON MONOXIDE CALIBRATION CURVES

Table 15: DETERMINATION OF CARBON MONOXIDE LEVELS IN THE RP/BR AEROSOL EXPOSURE CHAMBER

Study No.	Date Analyzed	Sampling Date /Sample Number	RP/BR Aerosol Mass Conc. mg/l[a]	Sample Size (torr)	Ave. Area	Relative Response	Concentration ppm(v)
35	2-24-83	2-4-83/2	1.10	250	34741	0.03762	5.23
	2-24-83	2-4-83/3	1.10	150	50406	0.03232	10.86
	3-1-83	2-4-83 Blank		150	92260	0.02559	15.80
45	2-24-83	2-23-83/1	0.59	250	70778	0.02814	7.97
	2-24-83	2-23-83/2	0.59	250	45545	0.03368	6.14
	3-1-83	2-23-83 Blank		100	80875	0.02714	21.95
48	2-24-83	2-25-83/1	1.07	150	54150	0.03139	11.33
	3-1-83	2-25-83 Blank		250	0.0	0.0	0.0
49	2-28-83	2-28-83/1	1.09	250	87654	0.02757	9.67
	2-28-83	2-28-83/2	1.09	250	68387	0.03048	8.34
	3-1-83	2-28-83 Blank		250	0.0	0.0	0.0
52B	3-15-83	3-7-83/1	2.12	250	115118	0.02420	11.14
	3-15-83	3-7-83 Blank		250	0.0	0.0	0.0
	3-16-83	3-15-83/2	1.04	250	42050	0.03467	5.83
	3-15-83	3-15-83 Blank		250	0.0	0.0	0.0
	3-15-83	3-15-83 Hallway Blank		250	0.0	0.0	0.0
	3-16-83	3-15-83 Blank at Meter Exit[b]		250	29418	0.04020	4.73
57B	3-16-83	3-16-83/1	1.02	250	44026	0.03402	5.99
	3-16-83	3-16-83/2	1.02	250	38862	0.03582	5.57
	3-16-83	3-16-83 Blank		250	0.0	0.0	0.0
57B	3-18-83	3-18-83/1	0.93	250	56238	0.02857	6.43
	3-18-83	3-18-83 Blank		250	0.0	0.0	0.0
57A	3-18-83	3-18-83/1	0.33	250	54191	0.02941	6.05
	3-31-83	3-18-83 Blank		250	0.0	0.0	0.0
64	3-31-83	3-29-83/1	0.54	250	71730	0.02780	7.98
	3-31-83	3-29-83/2	0.54	250	66769	0.0	7.63
	3-31-83	3-29-83 Blank		250	0.0	0.0	0.0

[a] Determined gravimetrically from filter-collected samples.

[b] Air exhausted from the exposure chamber through gas meter used to monitor air flow through the filter samples.

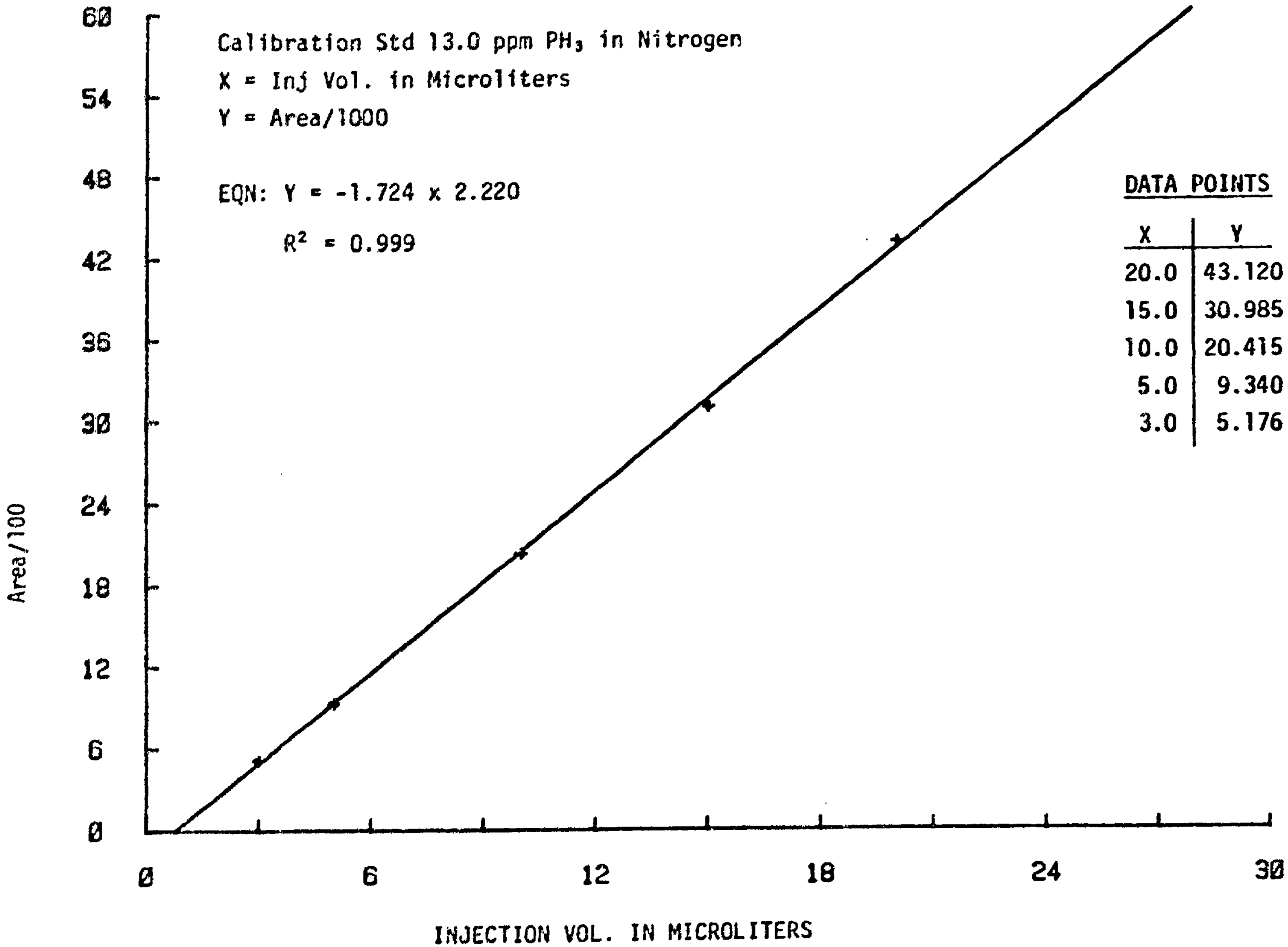


FIGURE 16. TYPICAL PHOSPHINE CALIBRATION CURVE USING N-P DETECTOR

Table 16: DETERMINATION OF PHOSPHINE LEVELS IN THE RP/BR AEROSOL EXPOSURE CHAMBER

Study No.	RP/BR Aerosol Mass Conc. (mg/l)[a]	Sampling Date	Sample No.	PH_3 Concentration ppb (v)
46B	3.09	2-24-83	1-C	<10
			2-C	<10
			Blank	0.0
48	1.07	2-25-83	1-D	0.0
			Blank	0.0
49	1.09	2-28-83	1-E	0.0
			Blank	0.0
57	0.93	3-18-83	2-J	0.0
57	0.33	3-18-83	2-K	<10
			Blank	0.0

[a] Determined gravimetrically from filter-collected samples.

3. Hexane

Three sets of exposure chamber air samples were obtained and analyzed for hexane in the 1 to 3 mg/l RP/BR aerosol concentration range. No hexane was detected in any of the samples above the minimum detectable limit of 1 ppm.

V. CONCLUSIONS AND RECOMMENDATIONS

Extensive statistical analysis of the pilot chamber revealed conditions of spatial and temporal homogeneity for filter and photosensor samples and for percent phosphoric acid levels. Although a statistically significant, spatial particle size gradient was found, this gradient did not affect homogeneity of aerosol concentration. More importantly, the change encountered was not significant biologically in terms of inhalation and deposition into the tracheobronchial region and the deep lung. Particle size distribution was homogeneous when measured over time. Although inspection of four additional chambers revealed some significant deviations from a statistical point of view these were under the 20 percent variation limits set for the homogeneity tests on the basis of engineering performance of the complex test article- generator-chamber system. Therefore, we concluded that conditions in these chambers were also homogeneous.

In reviewing the data, an interesting dichotomy is noted. The pilot chamber which has the most samples was homogeneous and the other chambers that have fewer samples were not statistically homogeneous. Since we are claiming that statistical power analysis suggests a gross oversampling, this finding would appear to be contradictory. This contradiction can be easily resolved. Statistical significance in this study is a function of three things: between replicate variability, between shelf variability and sample size. In the pilot chamber, between replicate variability was in fact larger than in the four other chambers; therefore, the ratio of between shelf to between replicate variability was small. This leads to nonsignificant F-statistics. In contrast, the additional chambers had extremely small between replicate variability, probably due to the increased experience of the technical staff when these chambers were tested, and therefore, the slightest shelf to shelf variability became statistically significant.

Thus our findings have clearly demonstrated the importance of choosing sample sizes so that statistical tests are only sensitive to deviations that are meaningful from an overall technical perspective. In future studies we therefore recommend conducting preliminary sampling experiments to be used for provisional statistical evaluation of spatial and temporal homogeneity. The important point is that sample sizes should be chosen appropriately in relation to the variability which is observed in the chambers and the power with which this variance can be measured. A number of factors enter into the attainment of adequate homogeneity. Some of these variables are the generators, the RP/BR billets, the exposure chambers, instrumentation, laboratory analytical methodology and personnel. On the basis of the suggested preliminary experiments a proper sampling size for a specific experimental design can be estimated more precisely by the investigators.

APPENDIX A

TABLES

Table A-1: PILOT CHAMBER (NO. 3) HOMOGENEITY STUDY: MEANS ±SD FOR AEROSOL MASS CONCENTRATION, PARTICLE SIZE AND % PHOSPHORIC ACID MEASUREMENTS AT THREE CONCENTRATIONS FOR ALL SAMPLING LOCATIONS

Target Conc mg/l	Position No.	Filter Samples			Photosensor Readings			Particle Size Mass Mean Aerodynamic Diameter, μm			% Phosphoric Acid		
		Mean	± SD	N	Mean	± SD	N	Mean	± SD	N	Mean	± SD	N
0.2 mg/l	1	0.35	0.12	3				0.27	0.04	3	68.24	22.06	3
	2	0.32	0.05	3				0.31	0.05	3	64.64	5.85	3
	3	0.35	0.08	3				0.29	0.02	3	63.98	11.33	3
	4	0.31	0.14	3				0.35	0.02	2	77.86	18.39	3
	5	0.31	0.06	3				0.29	0.05	3	64.14	6.41	3
	6	0.29	0.10	3	0.26	0.06	19	0.26	0.02	2	44.18	12.37	3
	7	0.39	0.15	3				0.29	0.03	3	52.21	12.75	3
	8	0.46	0.31	3	0.19	0.04	19	0.35	0.0	1	62.04	11.96	3
	9	0.21	0.03	3				0.32	0.09	2	70.34	19.06	3
	10	0.28	0.06	3				0.35	0.07	2	60.26	14.64	3
	11	0.36	0.09	3				0.34	0.05	3	54.36	12.43	3
	12	0.25	0.07	3				0.39	0.16	3	53.96	15.61	3
	13	0.28	0.03	3				0.33	0.11	3	71.70	6.51	3
	14	0.40	0.09	3				0.36	0.0	1	57.15	11.59	3
	15	0.31	0.05	3				0.33	0.02	3	68.68	15.03	3
	16	0.28	0.07	3				0.32	0.04	2	63.85	5.89	3
	17	0.32	0.08	3				0.33	0.09	3	60.92	6.31	3
	18	0.30	0.05	3	0.22	0.08	18	0.30	0.04	3	64.32	5.52	3
	19	0.33	0.10	3	0.28	0.08	19	0.30	0.0	1	62.44	10.61	3
	20	0.35	0.06	3				0.36	0.02	2	60.84	9.11	3
	21	0.34	0.13	3				0.35	0.06	3	53.71	8.12	3
	22	0.36	0.11	3				0.36	0.06	2	53.77	7.70	3
	23	0.32	0.05	3				0.35	0.01	3	64.95	5.77	3
	24	0.22	0.06	3				0.37	0.07	3	60.64	4.30	3
	25	0.31	0.05	18	0.22	0.05		0.32	0.07	21	63.37	6.61	18
	Overall Mean	0.32	0.09		0.23	0.07		0.33	0.06		61.98	11.20	

Table A-1 (Continued)

Target Conc mg/l	Position No.	Aerosol Mass Concentration, mg/l: Filter Samples Mean	± SD	N	Photosensor Readings Mean	± SD	N	Particle Size: Mass Mean Aerodynamic Diameter, μm Mean	± SD	N	% Phosphoric Acid Mean	± SD	N
0.5 mg/l	1	0.64	0.11	3				0.41	0.17	3	82.76	4.53	3
	2	0.58	0.11					0.40	0.15	3	60.05	4.22	3
	3	0.58	0.15	3				0.37	0.02	3	60.10	4.79	3
	4	0.66	0.03	3				0.35	0.01	3	56.31	13.27	3
	5	0.60	0.05	4				0.34	0.02	3	65.55	9.65	4
	6	0.65	0.03	3	0.51	0.06	21	0.36	0.0	2	60.85	6.89	3
	7	0.59	0.04	3				0.41	0.05	3	70.15	20.37	3
	8	0.55	0.07	3	0.50	0.05	21	0.44	0.06	3	63.29	3.68	3
	9	0.68	0.32	3				0.47	0.03	3	59.01	7.48	3
	10	0.56	0.04	3				0.33	0.06	3	46.76	23.92	4
	11	0.52	0.13	3				0.41	0.08	3	54.62	3.17	3
	12	0.65	0.30	3				0.39	0.03	2	60.08	6.06	3
	13	0.64	0.05	3				0.43	0.04	2	61.85	7.51	3
	14	0.59	0.13	3				0.46	0.06	2	56.38	5.91	3
	15	0.64	0.03	3				0.45	0.04	3	60.64	6.97	3
	16	0.63	0.01	3				0.48	0.02	3	61.33	5.45	3
	17	0.58	0.05	4				0.46	0.07	3	60.12	7.25	4
	18	0.58	0.07	3	0.45	0.09	21	0.43	0.08	3	72.92	10.83	3
	19	0.61	0.05	4	0.63	0.06	21	0.48	0.03	3	57.56	5.96	4
	20	0.57	0.15	3				0.50	0.01	3	57.38	4.94	3
	21	0.65	0.06	3				0.48	0.08	2	59.64	5.19	3
	22	0.64	0.07	3				0.46	0.06	3	65.91	15.40	3
	23	0.62	0.04	3				0.48	0.04	2	58.66	8.68	3
	24	0.53	0.03	3				0.49	0.06	3	53.55	10.06	3
	25	0.62	0.06	19	0.56	0.06	21	0.39	0.08	24	58.12	5.97	19
	Overall Mean	0.61	0.10		0.53	0.09		0.42	0.08		60.32	10.20	

Table A-1 (Continued)

Target Conc mg/l	Position No.	Aerosol Mass Concentration, mg/l: Filter Samples Mean	± SD	N	Photosensor Readings Mean	± SD	N	Particle Size: Mass Mean Aerodynamic Diameter, µm Mean	± SD	N	% Phosphoric Acid Mean	± SD	N
1.0 mg/l	1	1.15	0.04	3				0.49	0.06	3	62.26	5.54	3
	2	1.15	0.04	3				0.46	0.07	3	-	-	-
	3	1.06	1.16	3				0.48	0.06	3	67.87	7.27	3
	4	1.13	0.08	3				0.47	0.09	3	66.19	2.51	3
	5	1.15	0.08	3				0.43	0.03	3	-	-	-
	6	1.16	0.04	3	1.04	0.06	20	0.52	0.14	3	63.97	3.38	3
	7	1.02	0.01	3				0.53	0.06	3	60.82	5.47	3
	8	1.07	0.02	3	0.90	0.04	21	0.52	0.08	3	63.33	0.0	1
	9	1.02	0.03	3				0.48	0.10	3	62.96	4.39	3
	10	1.00	0.10	3				0.49	0.0	3	63.66	0.91	3
	11	1.03	0.04	3				0.50	0.07	3	-	-	-
	12	0.92	0.10	3				0.51	0.06	3	55.93	5.02	3
	13	1.16	0.04	3				0.55	0.08	3	54.87	19.82	3
	14	1.11	0.04	3				0.59	0.13	3	-	-	-
	15	1.10	0.02	3				0.55	0.06	3	64.06	3.69	3
	16	1.15	0.05	3				0.56	0.08	3	63.62	2.49	3
	17	1.05	0.08	3				0.53	0.05	3	-	-	-
	18	1.02	0.10	3	0.87	0.03	21	0.57	0.04	3	67.52	7.09	3
	19	1.05	0.12	3	1.13	0.06	21	0.57	0.04	3	60.24	3.23	3
	20	1.08	0.03	3				0.61	0.07	3	-	-	-
	21	1.10	0.03	3				0.58	0.06	3	62.87	9.11	3
	22	1.17	0.02	3				0.58	0.08	3	60.25	5.10	3
	23	1.07	0.06	3				0.62	0.06	3	-	-	-
	24	1.08	0.30	3				0.59	0.08	3	63.23	4.71	3
	25	1.08	0.13	3	0.99	0.07	21	0.53	0.14	19	64.52	4.18	18
	Overall Mean	1.08	0.11		0.99	0.11		0.53	0.08		63.07	6.13	

Table A-2: HOMOGENEITY STUDY FOR CHAMBER NOS. 1, 2, 4, AND 5: MEAN ± SD FOR AEROSOL MASS CONCENTRATION, PARTICLE SIZE AND % PHOSPHORIC ACID MEASUREMENTS FOR ALL SAMPLING LOCATIONS AT ONE CONCENTRATION FOR EACH CHAMBER

		Aerosol Mass Concentration, mg/l						Particle Size Mass Mean Aerodynamic Diameter, μm			% Phosphoric Acid		
		Filter Samples			Photosensor Readings								
Chamber No.	Position No.	Mean	± SD	N	Mean	± SD	N	Mean	± SD	N	Mean	± SD	N
1	1	1.13	0.03	3				0.42	0.04	3	72.66	12.15	3
	2	1.08	0.06	3				0.46	0.02	3	69.39	5.36	3
	3	1.12	0.09	3				0.40	0.04	3	72.78	2.21	3
	4	1.07	0.09	3	1.07	0.09	17	0.46	0.02	3	71.87	4.29	3
	5	1.12	0.09	3				0.48	0.01	3	66.64	0.0	1
	6	1.01	0.10	3	1.06	0.07	17	0.48	0.03	3	73.52	0.0	1
	7	1.09	0.07	3				0.51	0.04	3	72.63	0.0	1
	8	0.91	0.10	3				0.49	0.02	3			
	9	1.14	0.11	3				0.53	0.01	3	77.61	0.0	1
	10	1.09	0.09	3				0.57	0.02	3			
	11	1.16	0.07	3	1.07	0.08	17	0.57	0.02	3	76.55	0.0	1
	12	0.99	0.06	3				0.55	0.08	3	71.42	0.0	1
	13	1.18	0.04	3				0.57	0.08	2	69.38	9.82	3
	14	0.92	0.05	3				0.58	0.01	3	68.65	6.51	3
	15	1.02	0.06	3				0.59	0.03	3	72.94	1.74	3
	16	1.01	0.08	3	1.03	0.07	17	0.57	0.05	3	67.46	4.20	3
	17	1.00	0.09	18	1.11	0.05	18	0.52	0.05	17	69.74	10.04	16
	Overall Mean	1.04	0.10		1.06	0.08		0.51	0.06		70.64	7.39	
2	1	0.57	0.07	3				0.37	0.03	3	56.03	3.71	3
	2	0.53	0.03	3				0.38	0.02	3	53.09	2.80	3
	3	0.59	0.04	3				0.40	0.03	3	57.58	2.72	3
	4	0.60	0.03	3	0.52	0.04	18	0.35	0.06	3	58.83	3.33	3
	5	0.59	0.11	3				0.39	0.03	3			
	6	0.42	0.03	3	0.49	0.03	18	0.37	0.05	3			
	7	0.53	0.07	3				0.43	0.02	3			
	8	0.37	0.02	3				0.41	0.05	3			
	9	0.60	0.03	3				0.45	0.02	3			
	10	0.45	0.04	3				0.40	0.02	3			
	11	0.55	0.04	3	0.50	0.03	18	0.46	0.03	3			
	12	0.46	0.06	3				0.40	0.01	3			
	13	0.58	0.08	3				0.45	0.02	3	55.07	9.91	3
	14	0.40	0.03	3				0.40	0.04	3	50.79	7.37	3
	15	0.59	0.02	3				0.52	0.04	3	58.22	4.70	3
	16	0.44	0.05	3	0.50	0.03	18	0.43	0.01	3	61.34	8.85	3
	17	0.47	0.08	18	0.58	0.04	18	0.45	0.04	18	53.64	8.11	17
	Overall Mean	0.50	0.09		0.52	0.05		0.42	0.05		54.87	8.00	

Table A-2 (Continued)

Chamber No.	Position No.	Aerosol Mass Concentration, mg/l: Filter Samples Mean	± SD	N	Photosensor Readings Mean	± SD	N	Particle Size: Mass Mean Aerodynamic Diameter, μm Mean	± SD	N	% Phosphoric Acid Mean	± SD	N
4	1	0.28	0.90	3				0.29	0.02	3	64.92	5.62	3
	2	0.30	0.04	2				0.32	0.09	3	66.25	12.78	2
	3	0.29	0.06	3				0.26	0.03	3	63.50	6.82	3
	4	0.32	0.04	3	0.23	0.04	18	0.31	0.06	3	64.85	6.50	3
	5	0.33	0.05	2				0.32	0.02	3			
	6	0.25	0.03	3	0.20	0.03	18	0.28	0.05	3			
	7	0.30	0.06	3				0.33	0.08	3			
	8	0.23	0.04	3				0.31	0.01	3			
	9	0.32	0.06	3				0.32	0.08	3			
	10	0.26	0.05	3				0.31	0.06	3			
	11	0.28	0.05	3	0.24	0.02	18	0.33	0.01	3			
	12	0.26	0.01	3				0.35	0.09	3	61.88	0.0	1
	13	0.31	0.08	3				0.40	0.05	2	61.52	7.35	3
	14	0.26	0.03	3				0.38	0.04	3	58.99	10.66	3
	15	0.30	0.06	3				0.33	0.04	3	65.29	12.47	3
	16	0.24	0.04	3	0.22	0.04	18	0.36	0.07	3	61.40	6.84	3
	17	0.23	0.05	18	0.25	0.03	18	0.30	0.09	18			
	Overall Mean	0.27	0.06		0.22	0.04		0.31	0.07		63.16	7.42	
5	1	1.27	0.02	3				0.49	0.06	3	73.91	1.35	3
	2	1.22	0.03	3				0.62	0.09	3	70.91	3.37	3
	3	1.27	0.02	3				0.49	0.03	3	72.39	0.89	3
	4	1.24	0.02	3	1.02	0.11	18	0.62	0.06	3	72.25	2.35	3
	5	1.22	0.05	3				0.59	0.05	3			
	6	0.35	0.02	3	0.94	0.06	18	0.56	0.06	3			
	7	1.24	0.03	3				0.58	0.05	3			
	8	0.98	0.03	3				0.61	0.06	3			
	9	1.23	0.07	3				0.63	0.03	3			
	10	0.81	0.05	3				0.66	0.08	3			
	11	1.17	0.09	3	1.02	0.13	18	0.58	0.12	3			
	12	1.07	0.02	3				0.62	0.05	3			
	13	1.29	0.04	3				0.60	0.01	3	69.68	4.82	2
	14	0.76	0.11	3				0.69	0.06	3	70.81	2.46	3
	15	0.98	0.09	3				0.65	0.03	3	73.04	3.18	3
	16	0.83	0.07	3	0.99	0.23	17	0.72	0.06	2	68.29	6.57	3
	17	0.98	0.06	18	1.14	0.07	18	0.63	0.05	18	70.67	0.0	1
	Overall Mean	1.06	0.17		1.02	0.14		0.61	0.07		71.45	3.30	

APPENDIX B

SELECTED PHOTOGRAPHS FROM THE INHALATION EXPOSURE LABORATORY

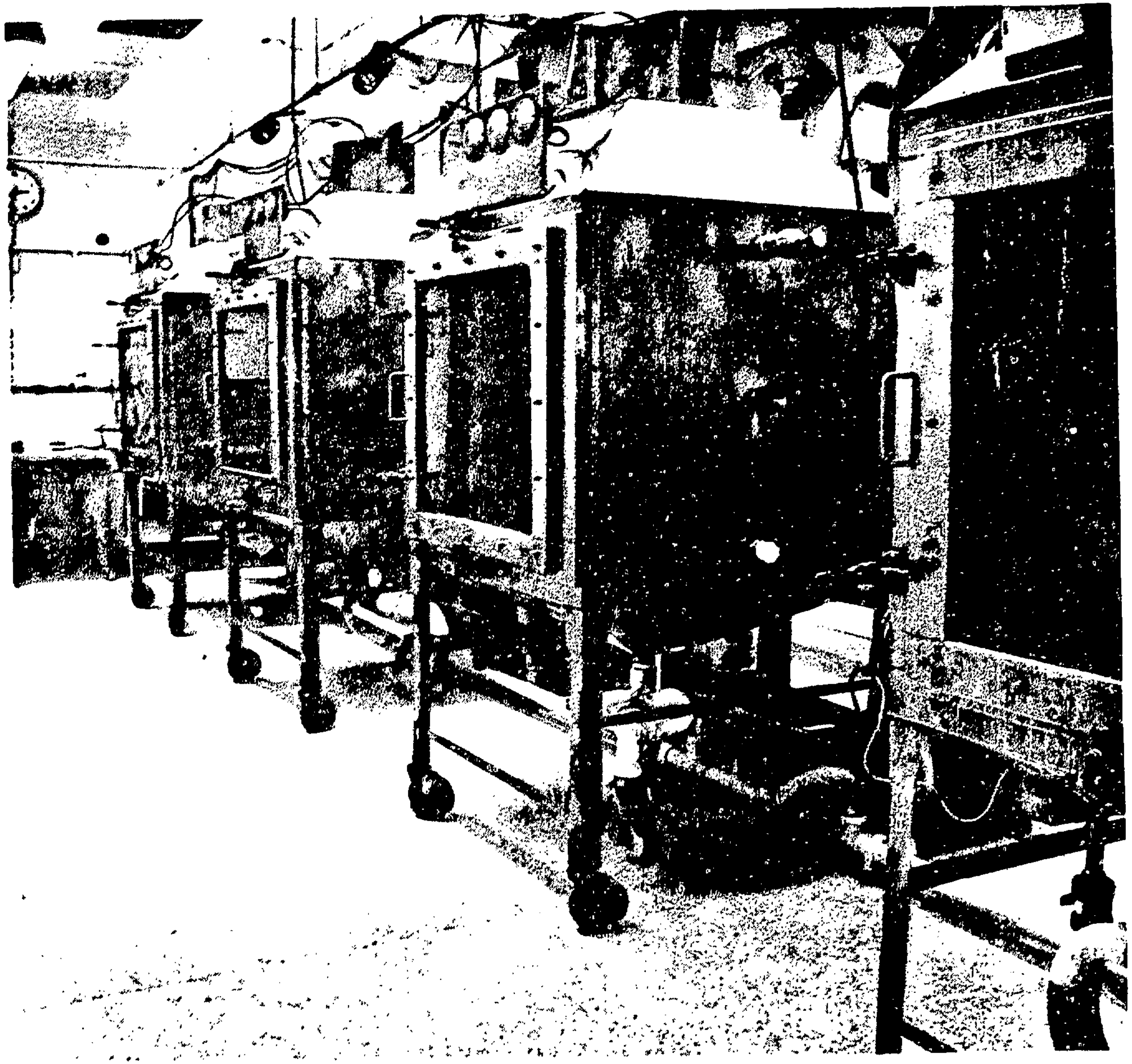


FIGURE B-1: INHALATION EXPOSURE CHAMBER FRONT VIEW

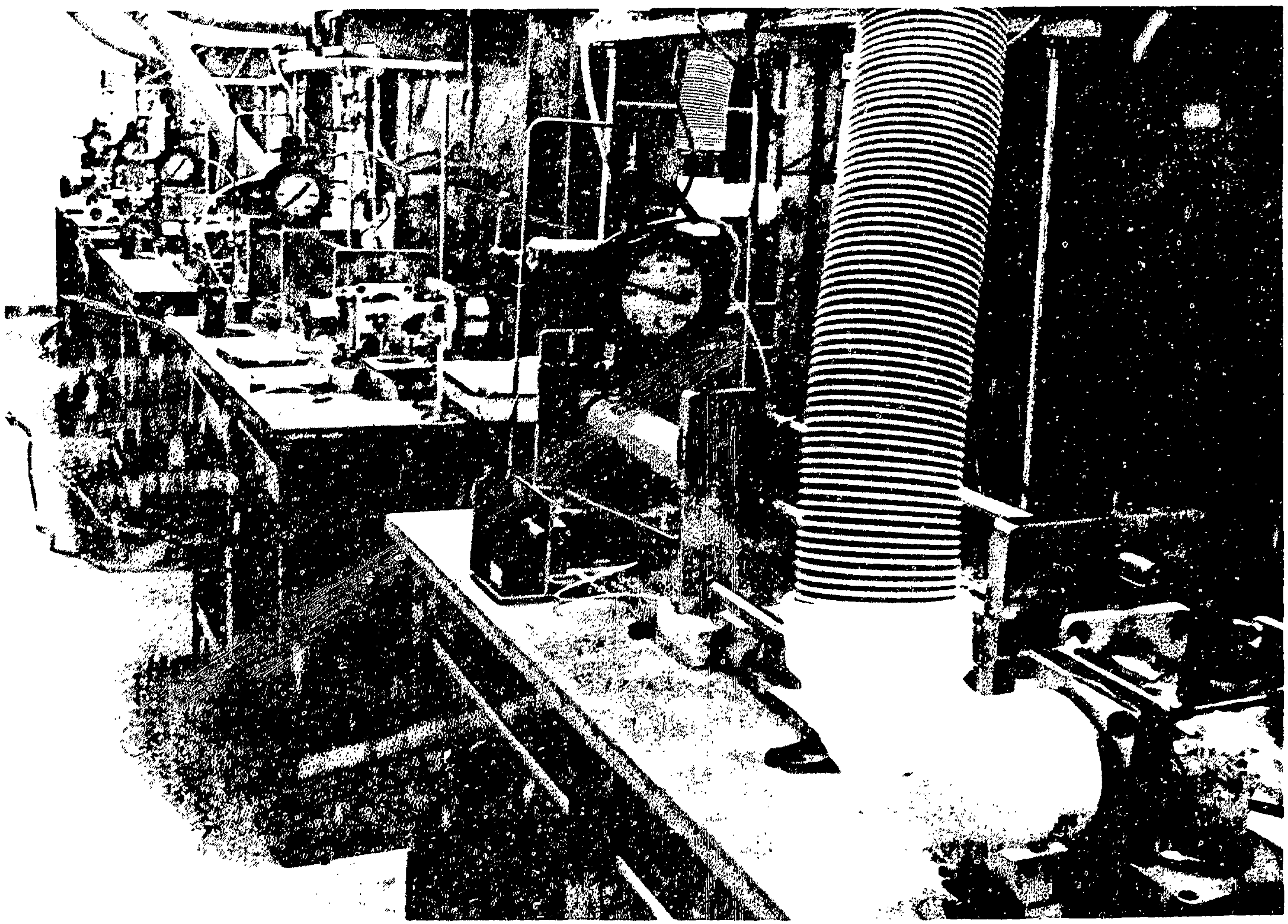


FIGURE B-2: AEROSOL GENERATORS SHOWN FROM REAR VIEW OF INHALATION EXPOSURE CHAMBERS

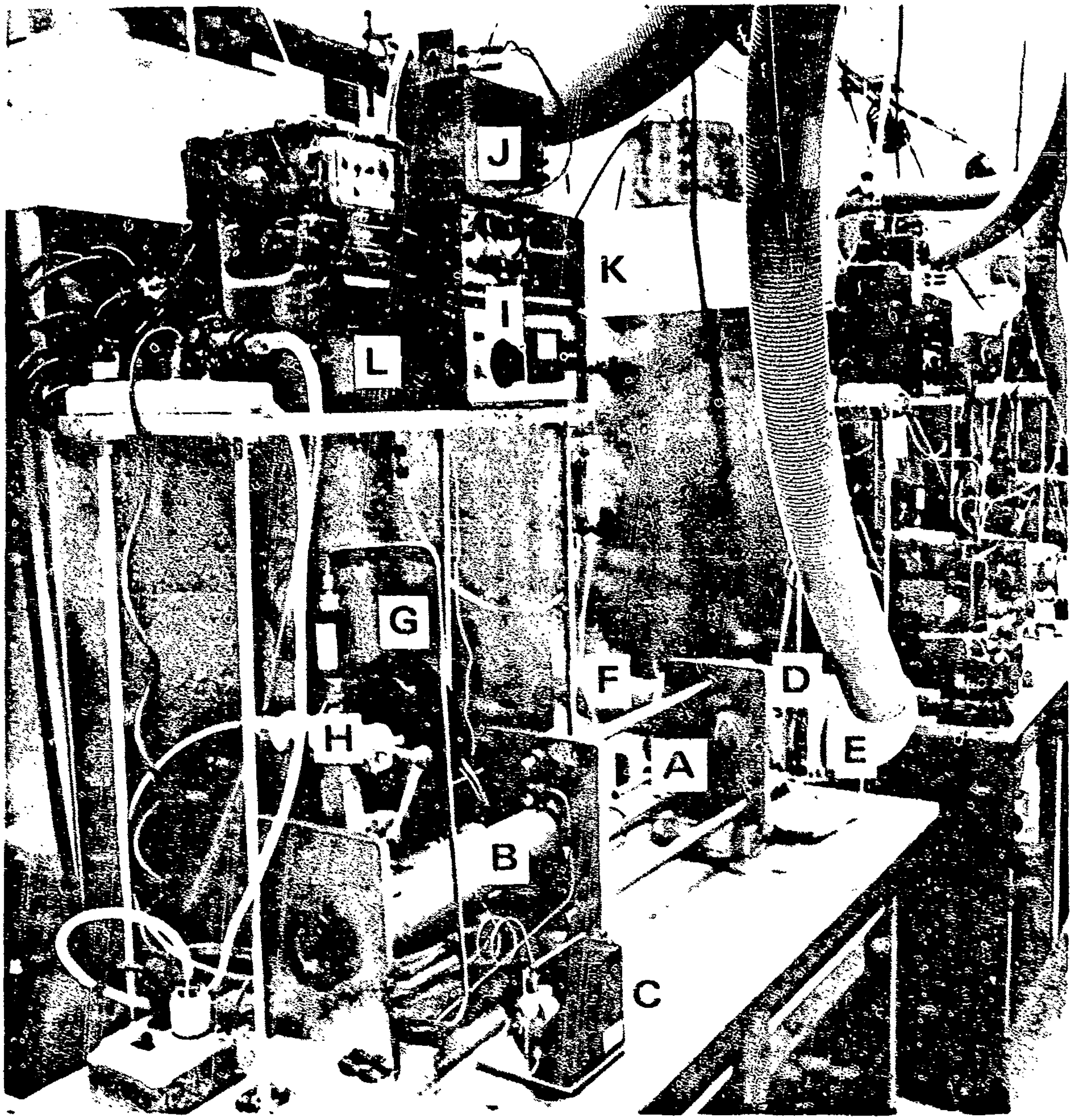


FIGURE B-3: AEROSOL GENERATOR AND MONITORING EQUIPMENT: *A extrusion cylinder and piston, B Hydraulic piston, C Precision metering pump, D Burn chamber, E Filtered air inlet, F Aerosol duct, G Extrusion pressure gauge, H Pressure safety valve, I Igniter power supply, J Photosensor alarm, K Photosensor amplifier of aerosol particle sensor, L Dry gas meter*

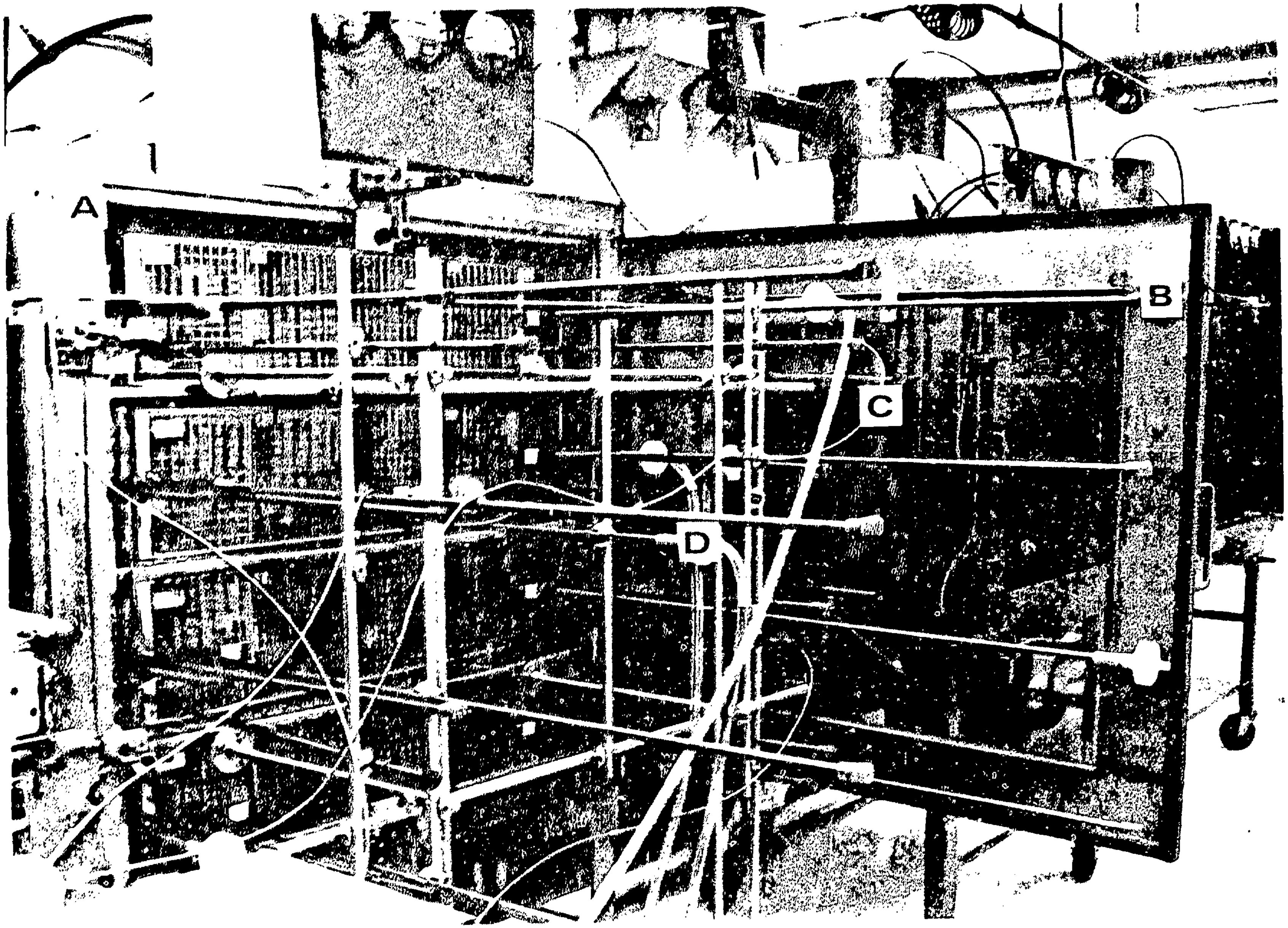


FIGURE B-4: EXPOSURE CHAMBER PREPARED FOR AEROSOL HOMOGENEITY TESTING: *A Plastic door replacement plate, B Sampling probe, C Photosensor, D Filter*

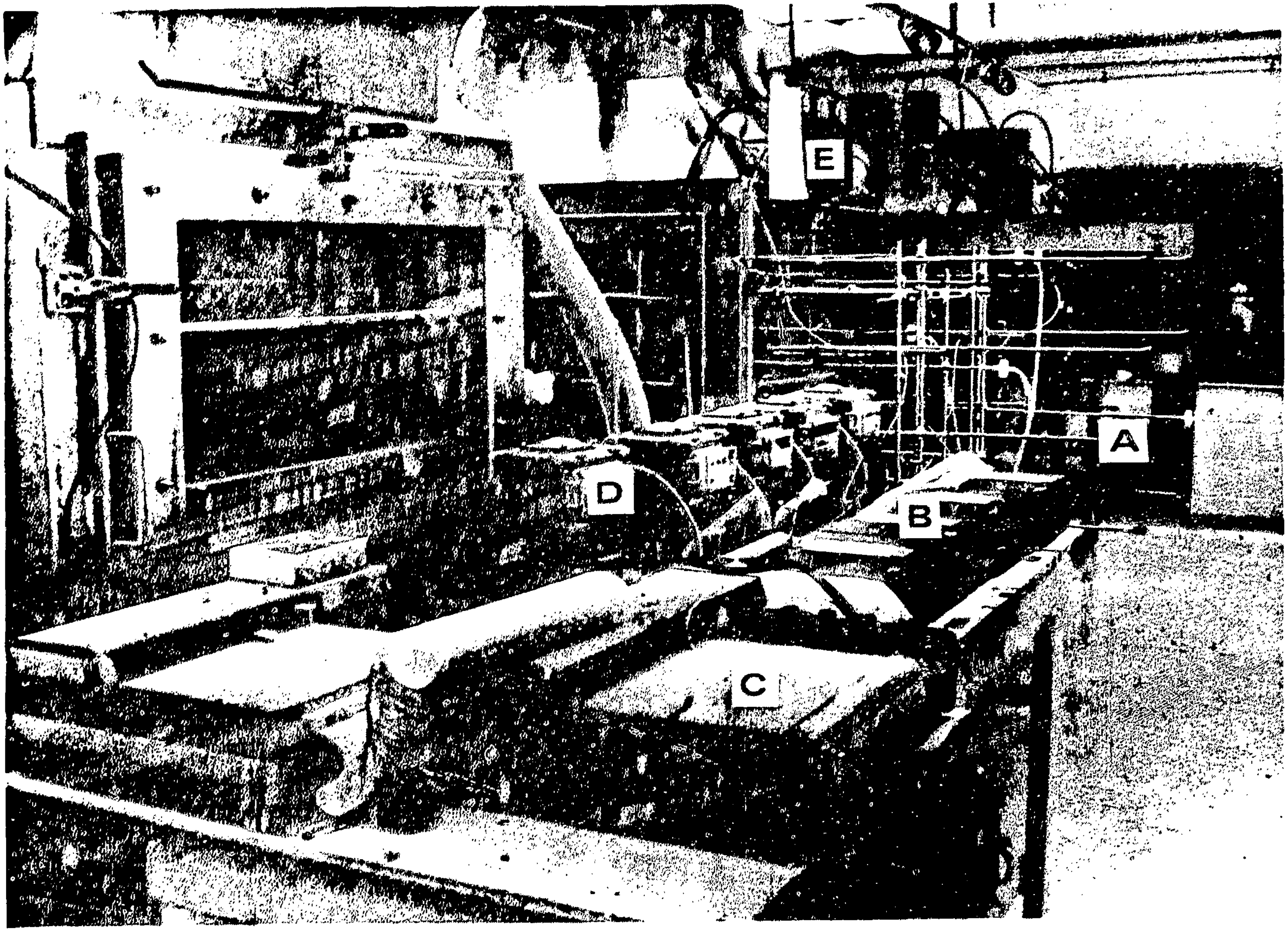


FIGURE B-5: CHAMBER WITH AEROSOL HOMOGENEITY SAMPLING EQUIPMENT: *A Aerosol sampling probe, B Photosensor amplifiers, C Strip chart recorders, D Dry gas meters, E Chamber pressure differential gauges*

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